VOLUME 42 FEBRUARY, 1956 NUMBER 1

The Journal of PARASITOLOGY

OFFICIAL ORGAN OF THE
AMERICAN SOCIETY OF PARASITOLOGISTS
FOUNDED BY HENRY BALDWIN WARD

EDITORIAL COMMITTEE

LEON JACOBS, CHAIRMAN
HAROLD W. MANTER
BERNARD V. TRAVIS

EDITORIAL BOARD

WILLIAM BALAMUTH
ELON E. BYRD
RAYMOND M. CABLE
G. ROBERT COATNEY
WILLIAM W. CORT
CHAUNCEY G. GOODCHILD
GLEN M. KOHLS
R. BARCLAY McGHEE
ALLEN McINTOSH
HORACE W. STUNKARD
PAUL E. THOMPSON
PAUL P. WEINSTEIN

OFFICERS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

President: Eloise B. Cram
President-elect: Gilbert F. Otto
Vice-president: Aurel O. Foster
Secretary: Arthur C. Walton, Knox College, Galesburg, Illinois.
Treasurer: Robert M. Stabler, Colorado College, Colorado Springs, Colorado.

Council members ex officio: Leon Jacobs, *Chairman, Editorial Committee.* Wilford S. Bailey, *Custodian, Back Issues.* Norman R. Stoll, *Custodian Endowment Fund.*
Council Members at Large: Raymond M. Cable, William Trager, Allen McIntosh, Donald V. Moore, Frances J. Kruidenier, Paul C. Beaver, Luther O. Nolf, Leslie A. Stauber.

Persons interested in any branch of parasitology may apply for membership in the American Society of Parasitologists. Those interested in membership are invited to write to the Secretary who will furnish membership application blanks. The annual dues for the Society ($6.00) includes a subscription to The Journal of Parasitology.

THE JOURNAL OF PARASITOLOGY

The Journal of Parasitology, published bi-monthly, is the official organ of the American Society of Parasitologists and as such it publishes the program of the annual scientific meeting, abstracts of the papers presented there, and minutes of the business meetings of the Society.

The Journal of Parasitology is a medium for the publication of the results of research in the field of animal parasitology. Manuscripts dealing with subjects in this field will be considered for publication, and accepted manuscripts will be published in order of receipt. A manuscript is accepted with the understanding that it is to be published exclusively in the Journal unless otherwise arranged in advance.

Articles submitted for publication may be sent to any member of the Editorial Committee or Editorial Board. Responsibility for acceptance of papers is divided in the Editorial Committee for the fields of helminthology, protozoology and entomology. Correspondence concerning the disposition or revision of manuscripts should be addressed to Leon Jacobs, Chairman of the Editorial Committee, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland.

Manuscripts submitted should be typewritten, double or triple spaced, with wide margins, and with tables, graphs, and illustrations in finished form for transmission to the printer. Title, author, institution, etc., go on a separate sheet. Each table requires a separate sheet. Footnotes are not encouraged. Such material can usually be embodied in the text. If footnotes are used, they should be numbered consecutively and typed on a sheet separate from the text. Authors are urged to emphasize simplicity and clearness in tables. When feasible, sharp, contrasty photographic reproductions of large plates and graphs should be submitted in place of the originals. In preparing plates care should be given to the effective arrangement of figures, to economy of space, and to the amount of reduction required. Figures should be arranged within an area five by eight inches, or multiples thereof. Legends for figures should be typed on a sheet, separate from the text, and containing no illustrations. Greek characters and symbols should be avoided, where possible, since they require hand composition, thus increasing the cost of publication.

The bibliography, double or triple spaced, should contain only those references actually cited in the text, and its style should conform to bibliographies in recent issues of the Journal. The Index Catalogue of Medical and Veterinary Zoology should be used as a guide for abbreviating the journals cited.

Since it is the desire of the Editorial Committee to publish all acceptable manuscripts submitted within the limited space available, authors are urged to present their results briefly, avoiding tables where possible. However, acceptability of a paper will be determined not by its length but by the amount and character of new information included and the form in which it is presented. Long articles, and those with an unusual number of tables and plates, can be published only with the financial cooperation of the author.

Reprints may be purchased at cost from the printer if they are ordered when galley proof is returned. A blank form accompanies the proof for this purpose.

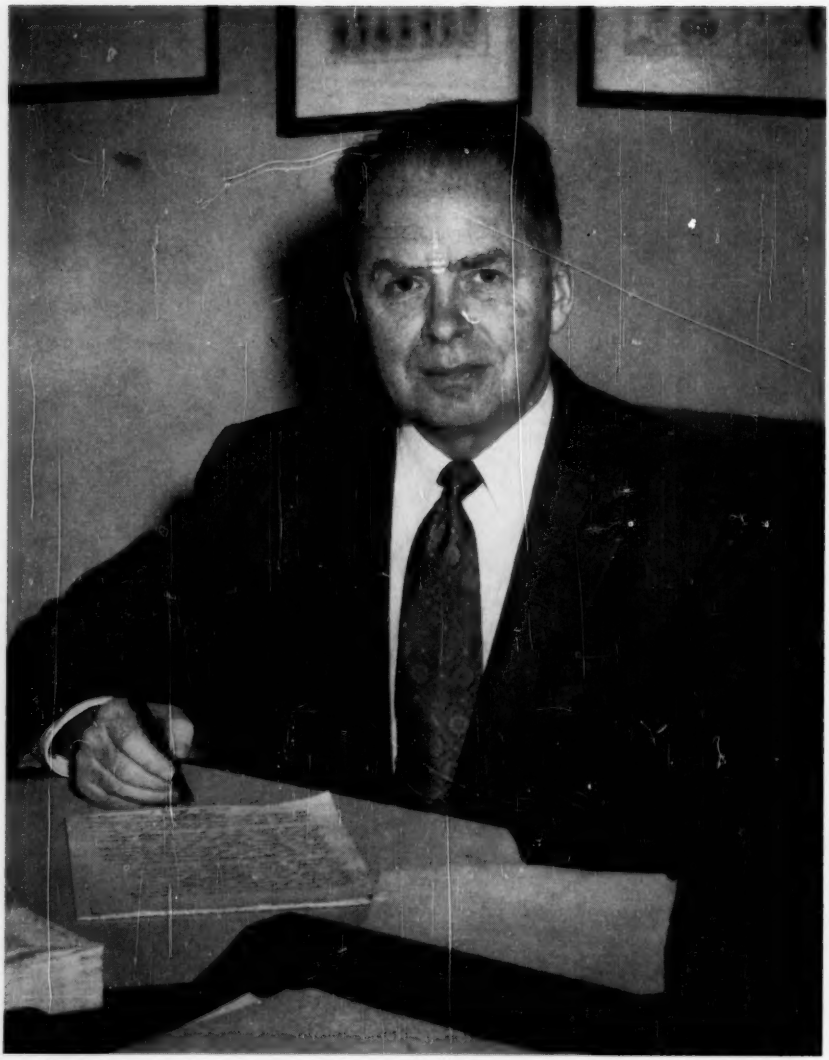
Claim for copies lost in the mails must be received by the Treasurer within sixty days following the mailing date. Delivery overseas cannot be guaranteed and must be at the subscriber's risk. Changes of address must be received by the Treasurer at least two weeks prior to the date of issue.

The subscription price for the Journal is $7.50 a volume (Foreign $8.00).

Correspondence concerning subscriptions and changes of address should be addressed to the Treasurer.

Correspondence concerning orders for back issues should be addressed to the Custodian, Wilford S. Bailey, Alabama Polytechnic Institute, Auburn, Alabama.

Entered as second-class matter December 21, 1932, at the post office at Lancaster, Pa., under the Act of March 3, 1879.



Clay L. Huff

The Journal of Parasitology

Volume 42 FEBRUARY, 1956 Number 1

PARASITISM AND PARASITOLOGY*

CLAY G. HUFF

Division of Parasitology, Naval Medical Research Institute, National Naval Medical Center
Bethesda, Maryland

Although parasitology is a department of knowledge, in its present stage of development it is a very young science. The field of study to which this name is usually applied is a conglomerate of many different aspects of the study of the animals which are dependent upon other animals for their sustenance; it includes systematics, natural history, physiology, biochemistry, immunology, treatment and control. I have, of course, not intended this to be a definition nor to exclude any of the unmentioned special interests which exist in this group of animals. Inasmuch as many of these parasites cause illness or death of their hosts it is easy to understand why so much of the time and energy of parasitologists has been spent in finding and describing them and then in trying to find means of preventing their entrance into or of removing them from their hosts. This portion of the development of our subject has added many illustrious pages to the history of human and veterinary medicine, of public health and tropical medicine. Likewise the great diversity of structure and the intricacies of the life cycles of parasites are sufficient justification for the large amount of energy and thought which have been expended in studying their interrelations with each other and their means of transferring from one host to another. These tasks are not finished. However, we should expect that a smaller proportion of the time, energy and thinking of parasitologists will need to be devoted to them in the future. It is my hope to point toward the development of a science of parasitology having as its primary purpose a study of the principles of parasitism.

The need for more emphasis on this phase of parasitology is made more apparent by an attempt to state any general principles of parasitism. How many can you name? What corresponds in parasitology to Mendel's law in genetics, to the law of mass action in chemistry, to the principle of conservation of energy in physics, to mention only a few other fields for comparison? Moreover, in spite of the great contributions of parasitology to the health and comfort of man and domestic animals, what general contribution has our subject made to the other sciences? I believe that I do not need to emphasize by further statements or questions the rather primitive state of the science of parasitism.

The phenomena included in the term "parasitism" occur at several levels,—the subcellular, cellular, organismic, and social levels. As I have indicated, the major portion of the thinking and energy of parasitologists has been directed heretofore at the organismic level. Parasitism at the social level has received little attention from

* Address of the retiring President of the American Society of Parasitologists, 30th Annual Meeting, Atlanta, Georgia, Dec. 29, 1955.

parasitologists. Excepting the work of a few ecologists, the effects of parasites on social structure and of social structure on parasites have not received serious study I shall not be so bold as to suggest that parasitism in the social organization of *Homo sapiens* is a subject which parasitologists should claim. It is reasonable, however, to expect that closer attention to the organisms acting as parasites, commensals and inquilines in colonies of social animals might suggest new lines of investigation in the study of parasitism at lower levels of organization. I am optimistic enough to feel that just as a study of the genetics of *Drosophila, Nicotiana, Paramecium Neurospora,* and *Escherichia* have contributed to our understanding of human inheritance, so through study of the phenomena of parasitism by, for example, *Trichinella, Schistosoma, Trombicula, Plasmodium,* and rickettsiae, principles will eventually be established which can be applied to many other forms and will finally give us a clearer insight into questions of parasitism of and by man.

Because my interest has centered in recent years around problems of parasitism at the cellular and subcellular levels I shall draw more upon them in the discussion to follow. Of course, I should not like to imply that the problems at these levels are more interesting or important than those at higher levels of organization. It should, in fact, be apparent that problems of parasitism at one level grade imperceptibly into those of another level.

So far, I have confined my remarks to the animals which serve as parasites. Certainly at the cellular level, and I am inclined to believe that at higher levels, one should study parasitism as a phenomenon without regard to the classification of either the parasitic organism or the organism parasitized, as animal or plant. Furthermore, although I shall emphasize this point further, it appears to me that everything is to be gained by including under our scrutiny all degrees of parasitism from that which is most harmful to the host to the most extreme cases of mutualism in which both partners profit from the association.

TOWARD A SCIENCE OF PARASITOLOGY

Although it appears likely that as the more pressing practical needs in the control and cure of parasitic infections are solved there will naturally be a shift in emphasis to a study of the general principles of parasitism, this shift in emphasis can be speeded up through our efforts. There must first be a greater desire and then a devotion to implement this desire on the part of parasitologists. Realizing that a complete blueprint of the step by step development of a science cannot be drawn in advance it does, however, seem reasonable to me to suppose that to some extent the direction we take is in our hands. What I have to say on this subject is only a feeble attempt to encourage a wider tendency to take stock of our deficiencies and to dedicate ourselves to overcoming them.

One of the first advantages to be derived from an emancipation from the demands for immediate results should be a greater freedom in the choice of the organisms which we study. We need no better example of the value of this freedom than that shown by the organisms which geneticists have chosen for the intensive study of the principles of inheritance. Let us imagine the state of development of genetics today if the guiding factor in the choice of organisms to be studied had been the need for knowledge of inheritance in particular animals and plants. By the choice of such animals as cattle, sheep, or domestic fowl the development of our knowledge of the

fundamental principles of inheritance would be only in its beginning stages. There were no practical advantages in sight when *Drosophila* was chosen as a subject for the laboratory study of genetics. An intelligent selection of the host-parasite combinations most likely to be successfully handled in the laboratory and to yield basic information on parasitism should be a more suitable way of proceeding than to make the kind of choice which is so often made—i.e., what organism is today causing the most disease. So often the choice is made upon the basis of the availability of funds for research. I admit that here the scientist is often met with forces too strong to resist completely. Nevertheless, there must be resistance on our part if we are to be the ones who control the direction and speed of our development. I have seen heartening examples of such resistance in other fields even to the refusal to accept grants which determine the particular aspect of a subject to be studied. I greatly admire a geneticist friend who happens to be interested in working with a group of animals which are commercially of minor importance. Much of his experimental work has been financed out of his meager salary. It should not be necessary in this year of 1955 to point out to administrators and others who have the responsibility for allocating funds the desirability of developing research independent of the dictates of current need but it *is* necessary and I suspect it will be necessary in 2000 A.D. also. Herein lies one of our responsibilities as scientists, namely, our responsibility for keeping people in general informed of the need for the most favorable environment in which research makes progress.

I should like to prevent a misunderstanding of my meaning by saying that I do not imply that there is some special advantage in choosing for study a parasite-host combination which has no economic importance. In fact, in many cases it may be that the parasitic combinations most favorable to discovering the principles of parasitism happen to be ones of great importance to man. Furthermore, in cases where, for one reason or another, one is compelled to work upon a particular parasite he may find it possible to exercise his free choice of the various aspects of the problem open to study.

Another advantage of a greater freedom to choose the host-parasite combination most suitable to one's purpose is that it opens up a much wider field for study by permitting the study of parasitisms irrespective of whether or not they produce harm to their hosts. Undoubtedly all the general principles governing the interrelationships of host and parasite extend through all degrees of parasitism to mutualism.

It would also seem to be a reasonable assumption that most is to be discovered about interrelations between host and parasite when very well adjusted partnerships are dissolved and the contributions to the partnerships are separately analyzed. I think of no better illustrations of these obligate relationships than those (1) of the intestinal flagellates of termites and of the cockroach *Cryptocercus* and (2) of the intracellular symbiotes of insects. The classic experiments of Cleveland who analyzed the interrelations of the former by the use of such methods as oxygenation, starvation, and high temperatures are too well known to all of us to describe here. These experiments extending over a period of more than three decades illustrate what great yields can be gleaned from studying host-parasite relationships so interdependent that the "parasite" has thus far resisted attempts at *in vitro* cultivation. Moreover, the apparently obligate relationships between the intracellular symbiotes of insects have been for a long time a challenge to the experimentalist. These inter-

relations are so close that it has not always been clear whether we were dealing with two organisms—host and parasite—or with intracytoplasmic inclusions of the host cells. The successful separation of cockroaches from their intracellular "bacteroids" by Dr. Marion A. Brooks (1954) and the study of the physiology of the aposymbiotic cockroaches represents a break-through in the fortified line seemingly impregnable heretofore to the experimentalist. This break-through was possible by means of the use of the antibiotic, aureomycin. Fain would I now prophesy a rich yield of knowledge in this field if we have the courage and enterprise to exploit the lead which has been given us.

Recognizing that all instances of parasitism are highly specialized ecological associations it is not surprising that the nature of each is dependent upon the types of organism serving as parasites and as host, whether the hosts are warm or cold blooded, whether the parasites are large, multicellular or small, unicellular forms, whether they are ecto- or endoparasites, and whether they live in body cavities, in the digestive or urogenital tracts, or in the body fluids, or intracellularly.

In the ectoparasite the importance of the non-living environment increases with the amount of time the parasite lives away from its host. The special senses are important in guiding it to its host. Consequently, the physiology of the special senses of the parasitic arthropods is, perhaps the keynote in understanding this type of parasitism. Although progress is being made in the study of the general physiology as well as in the sensory physiology of arthropods it now constitutes a small proportion of the research work done on parasitic arthropods, especially in the United States.

The endoparasite has substituted for its environment a living animal which is less variable and more immediately reactive than a non-living environment. Because of the reactivity of the host, knowledge of the chemical constitution of the parasite and its excretions become important in helping unravel the complex interrelations of the pair of organisms. There is a lesser role of the special senses of the parasite. However, it is well not to overlook the fact that many endoparasites have free-living stages in their life-cycles and that the sensory mechanisms which guide them to their hosts are as important as they are to ectoparasites. A study of these sensory mechanisms is a phase of parasitology which has been sadly neglected.

Intracellular parasitism represents a complexity of relationships of great interest not only to parasitology but to many other fields. Lying within the protoplasm of the host cell, the parasite assumes a new interest. It is here in closest contact with the source of its nutrition and at the same time subject to the catabolic action of the protoplasm of the host cell. The interchanges which necessarily take place between parasite and host protoplasm present much the same types of problems as are presented by the normal intracellular physiology. The relationships between nucleus and the extranuclear organelles, and between the various types of extranuclear organelles resemble in complexity the parasite-host cell relationship. Moreover, much the same type of problem is presented by other types of intracellular infectious agents: the rickettsiae, the bacteria, the bacteroids, the fungi, and the filterable viruses. The principles of intracellular parasitism when they are known are likely to apply to all of these infectious agents which are capable of living within the cell.

A look at the problems of intracellular parasitism gives us a very convincing demonstration of the need for help from the fields of biochemistry and physiology

of both the parasite and the host. However, this need is not peculiar to the problems of intracellular parasitism. Therefore, what I have to say on this subject has fairly general application to all cases of parasitism. To be able to cultivate a parasite apart from its host is an important step toward promoting the investigation of the capabilities of the parasite, physiologically speaking. Cultivation of the protozoan parasites has been a challenge for some time and successful cultivation has been achieved for several of them. There has not been as great effort directed toward the cultivation of the multicellular parasites.

Mere growth of a parasite away from its host is usually not accompanied by great gains unless it can be grown free from all other living cells. Whether or not axenic cultures of all parasites can be accomplished is impossible to say. However, even the unsuccessful attempt often reveals information fundamental to a better understanding of the physiology of the parasite. In many instances in which success at axenic cultivation has not been achieved one may profitably choose closely related organisms which can be grown in axenic culture, not on the assumption that the physiology of the two will be identical but that information will be obtained which has comparative value.

The keystone for progress in the biochemistry and physiology of parasites is, however, the development of chemically defined media for their cultivation. The rapid advances in our knowledge of the physiology of the free-living protozoon, *Tetrahymena,* following the development of a chemically defined medium for it, points to the possibility of similar progress in research on parasite physiology when chemically defined media are found upon which they can be grown. Again, a promising approach with such techniques may be through studying free-living forms which are closely related to the parasitic forms or the free-living stages of parasitic species in axenic, chemically defined cultures.

One of the greatest handicaps in studying true parasites is the fact that they are hidden from our observation during a very large proportion of their life cycles. Population studies made at intervals of time have been the chief means for evaluation of their growth, multiplication and death. I have often likened our problem in studying parasites to a situation in which one wishes to study an insect which is harmful to corn. Given a field of corn which is free from the parasite, one may walk along the side of the field releasing some of the insects to be studied. One must then wait until several generations of multiplication have occurred, then in every 50th row one removes a few stalks of corn and examines them for the insect. Finally when the entire field has been invaded one is allowed to go into the field and attempt to piece together the events that have taken place by studying the effects produced in different portions of the field. While this is not a very good analogy it does point up the fact that our research is limited to only certain types of study. The sampling technique, if done at frequent enough intervals, may reveal changes in morphology, in localization in different parts of the host, and in the size, variability, and reproductive rate of the parasites. The behavior characteristics of the individual parasite and the nature of its inheritance are hidden from view.

The rich variety of genetic mechanisms found in free-living and in the less obligate of the parasitic microbes has been wonderfully demonstrated in the Symposium on Sex in Microorganisms at the Philadelphia meeting of the American Association for the Advancement of Science (1954). However, the genetic mechanisms of obli-

gate parasites are practically unknown. Their discovery and analysis await better methods of study. Great strides have been made in the analysis of cytoplasmic mutation and adaptation in several of the non-parasitic microorganisms. We can be fairly certain that similar mechanisms exist in parasites. If this be so, it is easy to understand that such ability to change must greatly alter the interrelations of the parasite with its host during the course of infection. For example, I have pointed out (1954) that the capacities of malarial parasites for living in various types of host cells follow definite patterns which change as the infection progresses. As yet, technical difficulties interfere with an elucidation of the nature of this change but it stands as a challenge to us. Although technically the problem is a more difficult one than the study of transformations in free-living protozoa, the more rapid development of our knowledge of the latter will undoubtedly lighten the burden of the task in parasitic microorganisms. The relationships between enzyme adaptation and cytoplasmic mutation among yeasts and bacteria are suggestive that similar possibilities hold for the organism which must meet the changing environment of its host.

It seems likely to me that the general principle utilized by Dr. Algire and his associates (1954) wherein cells are grown within permeable chambers inside the host offer considerable promise for the study of the individual parasite and the rules regulating its inheritance. By modifications of this method it should be possible to keep parasites physically separated from but chemically intimately associated with the living host so that the individual parasite—and in some instances its offspring—can be examined at will. Such devices may make possible the mating of selected males and females, the isolation of hermaphroditic females, and many other problems which are not at present feasible. It should also be possible by such methods to follow the fate of individual parasites in hosts with various degrees of natural or acquired immunity. Since membranes of different pore size can be manufactured it should also be possible to prepare some chambers from which the host cells are excluded and others which will permit the entrance of the host's cells.

One of the handicaps suffered by parasitologists is a mental one. Once the normal behavior of a parasite has been discovered there is a very general tendency to accept this as the only behavior possible for it. Diagrams of life-cycles and expressions describing the usual behavior often prevent one from attempting to do the unusual thing experimentally. In the early history of protozoology there was a tacit assumption that parasitic protozoa could not be cultivated. Many such assumptions are firmly rooted in our thinking yet today. Let me give one illustration. Until a few years ago, it was assumed that the malarial zygote must pass through and develop on the outside of the stomach wall of the mosquito. Weathersby (1952) demonstrated that exflagellation, fertilization, and complete sporogony could occur in the hemocoele of the mosquito and that oocysts bearing viable sporozoites could grow in many different locations in the mosquito. Experiments such as this are not just stunts. They tend to point the way toward experiments which otherwise are dismissed as impossible and will eventually lead to a better understanding of the evolutionary history and potentialities of the parasites studied. This mental handicap which causes us continually to underrate the potential capacities of parasites is one which each parasitologist can do something about, not only in his own experimental work but in his teaching. No scientist intends to keep Sacred Cows but

most of us wear glasses which either filter out the images of Sacred Cows or transform them into the everyday kind which appear only to feed in our pastures and regularly to supply us with important articles of our diet.

If our science of parasitology would appear to be in a discouragingly primitive stage of development, part of the blame may be put upon the extraordinarily difficult problems which stand between us and a flowering similar to what has already been witnessed in the other fields of science. However, we ourselves must shoulder much of the blame for our failure to dedicate ourselves to the long range goal of developing principles instead of entertaining ourselves with the bewildering array of forms, interrelations, and intricacies which could be revealed by any haphazard dipping into the field. Not only do we need to dedicate ourselves to a new goal but we must see to it that the generation of parasitologists now being trained shall begin their task adequately prepared in all the contributing disciplines and ardent with the prospect of rich rewards that will come from lives devoted to such a worthy ideal. Let us hope that each succeeding generation of parasitologists will come nearer to understanding parasitism.

THE SCIENTIST AND HIS PUBLIC RELATIONS

If I now appear to stray into forbidden territory it is because I feel that the need to do so is urgent. I am not so much concerned about the direction which our science will take as I am about the general atmosphere in which all science finds itself. We face the paradoxical situation that we live in a culture more dependent than any previous one upon science and its applications yet, in which the forces of anti-intellectualism are rampant. In our present culture, it is the rule that scientists are on the defensive, and that to be classed as an intellectual is often reason to be ridiculed. This is an unhealthy situation from which our society must recover or be doomed. To cure an illness, one must find its causes and combat them. There are undoubtedly numerous causes for the antagonisms now shown toward science and other intellectual pursuits. However, it would appear to be safe to assume that much of it is the result of lack of understanding on the part of society of the motivations of scientists, their emotional as well as intellectual attitudes, and of their ethics. We may feel that we as individuals cannot oppose the strong current of antiscientific influences which surround us. Shall we assume a fatalistic attitude and, like Micawber, wait for "something to turn up?" If misunderstanding is at the base of our difficulties, it seems to me that we, individually and collectively, can help to overcome it.

If the general public misunderstands scientists and the scientific way of life it is because communication has broken down. In this we bear a greater share of the burden of blame than society. Scientists are not essentially different from other people except that they understand the beauty and nobility of a way of life not generally understood. How can others see that beauty and nobility unless we do our utmost to help them to see and understand it?

In the earlier days of scientific endeavor much of our science was largely natural history. The subject matter was not far removed from the experiences of the general public. Moreover, many of our naturalists developed to a high degree the art of presenting his material to the public. Their writings and lectures were very popular. Even in those days the public considered the naturalist as, perhaps, a little

queer but they understood what he was doing much better than the public understands what is being done in science today. Then in too short a period of time for the public to adjust to it, there came the very rapid development and specialization of science and the concomitant withdrawal of the scientist into his ivory tower. The subject matter, the methodology, even the discoveries themselves became more difficult to convey easily to the nonscientific public. At the very time when the scientist should have been giving more attention to ways and means of keeping the general public informed he gave over less and less of his time and effort to this very important function. The result has been that scientists have lost the sympathetic understanding of all but a small proportion of the public. Again I emphasize that the individual scientist is not wholly to blame for this loss of contact but the onus of the task of reestablishing good public relations devolves upon him.

I confess that I have no blueprint for regaining the sympathies of our society for scientists. I put the following suggestions forth for whatever they may be worth, hoping that they will be the stimuli for the proposal and implementation of far better ones on the part of many of us. It seems to me that we must sacrifice some of our cherished isolation and let the public know more about us as human beings. They must come to see that the present concept of a scientist as being a remote individual, coldly intellectual and dispassionate, is a myth. We must let them know more about the human side of our lives; that far from being dispassionate the true scientist is passionately devoted to the quest for truth; and that in the scientific way of life there is much that can be absorbed by the non-scientist. By all of the means at our command we must share our inner thoughts, aspirations and ideals with our fellow men. First, we have the written and spoken word. Why should not the general public be better informed by us on the real problems of parasitology and how these touch their daily lives? The excellent popular treatise by Rothschild and Clay, "Fleas, Flukes, and Cuckoos" (1952), is one type of approach. Without insulting the intelligence of the nonparasitologist, these writers give a sympathetic insight into some of the problems of parasitology and help the reader to see that both endo- and ectoparasites may be more than abhorrent creatures. The popular lecture so effectively used by naturalists in former days could still be employed by those gifted in this art. Surely we have not exploited to the fullest extent the use of radio, motion pictures, and television in revealing the beauty and interest of the multiplicity of the parasitic (including the mutualistic) relationships among animals. More important, however, is the need for portraying the lives of everyday scientists. The biographical and fictional novel is still one of our most powerful means for forming public opinion. Too few great novels have sympathetically dealt with the common scientist. Too often the portrayals have been extreme; either in raising the scientist to the status of a superman or debasing him with ridicule. We cannot all be successful novelists but most of us can do a bit of writing of a more humble sort here and there which will serve much the same purpose as a novel.

And what about poetry? Next to music this is probably one of the best ways of portraying the inner life of the writer. Not many parasitologists have seriously attempted it. Contemporaries of one of our former presidents will remember with warm satisfaction the delightful, earthy, expressive poems which flowed from his pen. Dr. Maurice C. Hall will long be remembered for the human touch which he supplied to parasitological poetry. Is the parasitic life too prosaic for expression in poetic form? Read the poem by my friend, Karl P. Schmidt (1945), on the

second law of thermodynamics if you think that poetry must be limited to nonscientific subjects!

If, however, we are to strive to let our fellow men see into the lives of scientists we must make sure that what he sees will be worthwhile. What contributions can science make to ethics and, therefore, to the cause of good will among men? Not being a philosopher, I shall not attempt an involved philosophic reply. However, all of us realize that the greatest attraction that science has to offer is its dominant quest for truth. Honesty and sincerity are necessary prerequisites to this ideal. Unfair competition in this quest is abhorrent. The "let live" philosophy epitomized in the Golden Rule is essential to a healthy climate for science. Also the disciplinary effects of repeated failures in the face of earnest attempts, the perversity of parasites compared with which the perversity of inanimate objects is almost negligible, and the heartrending reverses which accompany any study such as parasitism lead—in the true scientist—to humility. Devotion to truth, honesty, sincerity, tolerance, and humility! Are these not the basis for a universal ethics? These transcend race, religion, nationality and political bent. If these are the legitimate attributes of the serious pursuit of the scientific way of life, what right have we to allow the general public to remain unaware of the fact? These attributes are the rewards which should be emphasized rather than the physical results from applied science which may contribute to man's well being or be used in his extermination. When it is well known that these are rewards which scientists seek, our existence will be justified in the eyes of society.

If, however, we accept this view, we have some housecleaning to do. Scientists cannot take an amoral point of view but must be swift in punishing flagrant abuses of our basic ethics. Dishonesty and intolerance are, at best, breaches in the etiquette of science to be punished by our stern disapproval if not by more severe measures. On the positive side it is, perhaps, more important that in training young persons for the pursuit of science we make a supreme effort to discourage from entering this profession any incurably dishonest persons no matter how brilliant they may be. Perhaps, we should also not expect that the younger student can appreciate the ethical values of science from our implicit actions alone but that occasional explicit discussions of these values may be required.

In summary, I have tried to indicate what some of our attitudes as parasitologists should be toward developing a science, the chief purpose of which will be an understanding of the phenomena of parasitism. I have outlined some of the requirements, as I see them, for development in this direction. As members of the community of scientists, I see a still greater challenge to us. We must help the general public toward a better understanding of us as human beings and of our motivations. At present, a "cold war" exists between the public and the community of scholars of which we, as scientists, are a part. I see the need for a more responsible attitude on the part of the scientist; first, toward keeping our own moral house in order and second, toward enlightening the public of our aims and motivations. Only when these tasks have been accomplished can we expect a more sympathetic attitude toward us and our work.

References

ALGIRE, G. H. 1954 Visualizing cellular behavior *in vivo*. XV Cong. de la Soc. Internat. de Chir. Bruxelles.

———, WEAVER, J. M., AND PREHN, R. T. 1954 Growth of cells *in vivo* in diffusion chambers. 7. Survival of Homografts in Immunized Mice. Jour. Nat. Cancer Inst. **15**: 493–507.

BROOKS, M. A. 1954 Certain aspects of the histochemistry and metabolic significance of the intracellular bodies (bacteroids) of cockroaches (Blattariae). Dissertation. University of Minnesota. 64 pp.

HUFF, C. G. 1954 Changes in host-cell preferences in malarial parasites and their relation to splenic reticular cells. Jour. Inf. Dis. **94**: 173–177.

ROTHCHILD, M. AND CLAY, T. 1952 Fleas, Flukes and Cuckoos, A Study of bird parasites. Philosophical Library, New York xiv + 304 pp.

SCHMIDT, K. P. 1945 Reflections on energy and entropy. Sci. Mon. **61**: 172.

WEATHERSBY, A. B. 1952 The role of the stomach wall in the exogenous development of *Plasmodium gallinaceum* as studied by means of haemocel injections of susceptible and refractory mosquitoes. Jour. Inf. Dis. **91**: 198–205.

WENRICH, D. H., LEWIS, I. F., AND RAPER, J. R. 1954 Sex in Microorganisms. Amer. Assoc. Advance Sci., Washington, D. C. v + 362 pp.

RESEARCH NOTE

THE PIG AS A HOST OF *SCHISTOSOMA MANSONI* IN PUERTO RICO

In Puerto Rico pigs are often tied to stakes placed at the margins of streams, with the result that these animals spend considerable time in the water. Some of these streams support colonies of *Australorbis glabratus* that have a high incidence of *Schistosoma mansoni*. Kuntz (1955, Amer. Jour. Trop. Med & Hyg., **4**: 383–413) lists several mammals which serve as hosts for this parasite. Pigs were not included and it has been reported by Rodriguez-Molina (1940, P. R. Jour. Pub. Health & Trop. Med., **15**: 362–372) and Pinto (1944, Mem. Inst. Oswaldo Cruz, **40**: 209–340) that they had been unable to recover eggs from pigs exposed to large numbers of cercariae. These investigators apparently did not autopsy the pigs exposed. It was felt that additional study of the possible relationship of this animal to the epidemiology of *S. mansoni* was warranted.

Two pigs, 40 and 50 days of age, were obtained from sources where there was little likelihood that they had been exposed previously to *S. mansoni* cercariae. The younger pig was exposed once to 3550 cercariae, from 10 or more snails, by immersing the posterior half of the body in the suspension for 45–60 minutes. Over a period of three weeks the older pig was exposed in the same way to 10,000 cercariae on eight different occasions. Both experimental animals exhibited signs of severe irritation during the course of the infection and immediately afterward. This was especially true of the animal receiving multiple exposures, and usually a rash was visible on the abdomen and proximal region of the hind legs on the day following exposure.

In the case of the former animal, examinations of the feces by acid-ether concentration and miracidial hatching methods were all negative up to 68 days after exposure. At that time the animal was autopsied. Five adult worms, one male and four females, were recovered from the mesenteric veins. Viable and dead eggs were found in the liver, intestinal wall, and mesentery. The other animal also failed to show any evidence of *S. mansoni* infection upon stool examination. It was killed on the 83rd day after the initial exposure. No adults were found, but a large number of dead eggs, and a few viable ones, were observed in the liver. In both cases the dead eggs were heavily encapsulated.

The microscopic examination of 35–40 tissue samples obtained from pigs at the abatoir at Caguas, a highly endemic area, failed to show any eggs of *S. mansoni*. The results indicate that if any eggs were present, their numbers were small. At this abatoir the blood, feces, and pieces of viscera were flushed off the floor and drained directly into the Rio Caguitas. If viable eggs were present they would have had an opportunity to hatch in the vicinity of the snail vector. The incidence of *S. mansoni* in the snails collected below the abatoir was relatively high but other sources of infection were present.

The data obtained indicate that *S. mansoni* can reach maturity and produce viable eggs in the pig, but that it is a poor host. There appears to be little likelihood that this animal plays a role in the transmission of schistosomiasis mansoni in Puerto Rico.—G. T. RIGGIN, JR. AND LUIS A. BERRIOS, *Tropical Research Medical Laboratory, U. S. Army, San Juan, Puerto Rico.*

ELECTRON MICROSCOPE OBSERVATIONS OF *TRYPANOSOMA EQUIPERDUM*[1, 2]

EVERETT ANDERSON, L. H. SAXE* AND H. W. BEAMS
Department of Zoology, State University of Iowa, Iowa City

A number of reports deal with the electron microscopy of unsectioned specimens of the genus *Trypanosoma:* Lofgren (1950), Kraneveld, *et al.* (1951), Meyer and Porter (1954). It is the purpose of the present paper to report some observations, made with the electron microscope, of ultra-thin sections of *Trypanosoma equiperdum.*

MATERIALS AND METHODS

Trypanosoma equiperdum used in this investigation was obtained from rats serially inoculated with the blood of an infected guinea pig made available through the kindness of Dr. D. B. McMullen of the Army Medical Service Graduate School. Blood was drawn from the heart into a 10 ml. syringe containing about 1 cc. of 6% solution of sodium citrate and subsequently delivered into a centrifuge tube and centrifuged three times. The first centrifugation was carried out for approximately 8 minutes, after which the plasma containing white cells and trypanosomes was decanted into a second tube and centrifuged for about 10 minutes, thereby throwing down the white cells and platelets. The serum after the second centrifugation was decanted into a new tube and centrifuged for the third time for approximately 20 minutes. After the third centrifugation, the supernatant containing the trypanosomes was delivered into another tube containing about 1 cc. of 1% osmium tetroxide solution (fixative) buffered at *p*H 7.25 according to the method of Palade (1952). The organisms were fixed for 20–30 minutes, washed, dehydrated, infiltrated and embedded. Infiltration was carried out using a methacrylate monomer mixture consisting of 72% N-butyl methacrylate and 28% methyl methacrylate with a catalyst added (Luperco, 0.2 gm./10 cc.). The infiltrated organisms to be embedded were put into number 4 gelatin capsules and placed in an oven and allowed to polymerize for 8 hours at 48° C.

The embedded specimens were sectioned with an International Minot rotary microtome, approximately 0.025 micron thick, and ribbons so obtained were mounted on copper-mesh grids previously covered with a supporting film prepared from a 2% solution of celloidin in amyl acetate.

Observations were made, without removing the embedding mixture, with an RCA model EMU-2B electron microscope. The electron micrographs were taken at an original magnification of 3700 or 5700 and thereafter enlarged photographically as desired.

Received for publication, August 22, 1955.
[1] Aided by Grant No. B-301 (C) from the Division of Research grants, National Institutes of Health.
[2] Thanks are due Mr. Newtol Press for technical assistance and Dr. T. C. Evans for kindly allowing us the use of the electron microscope which was purchased by a grant from the Iowa Division of the American Cancer Society.
* Present address: Department of Pharmacology, Medical School, West Virginia University, Morgantown, W. Va.

DESCRIPTION OF RESULTS

Blepharoplast. Because of its position, size, and structure the body illustrated at B in Figures 3 and 5 is interpreted as the blepharoplast. Occupying the central part of this body is seen, cut in cross section, the root of the flagellum with its 11 fibrils. The denser material surrounding the flagellum constitutes the matrix of the blepharoplast.

It is not clear what the nature of the body at Z in Figure 2 is. It does not seem to be a blepharoplast since its structure is considerably different from the blepharoplast in Figures 3 and 5, B. It may represent an unusually large vacuole, or perhaps more likely, a fold in the body wall similar to that seen cut in cross section at G in Figure 7. Such a condition could be produced by a fold in the membrane, due to a twisting of the organism. This interpretation is supported by the fact that it is not a constant structure and has no counterpart in light microscope preparations.

Kinetoplast. Just posterior to the blepharoplast is a rod-shaped body, the kinetoplast (K, Figs. 6, 7 and 8) which appears to be limited by a membrane (Fig. 6). Internally the kinetoplast is composed of a number of electron dense lamellae arranged at right angles to the longitudinal axis of the organelle. The lamellae are approximately 360 Å in thickness and surrounded by a clear, perhaps vacuolar, area. Whether or not this clear area is in part artifact is unknown. In Figure 6 the lamellae appear to be connected to one another by fine fibrils, much in the same manner as the chromomeres are connected in salivary gland chromosomes of *Drosophila.* Two of these organelles, probably the result of division are shown in Figure 7.

Flagellum and Undulating Membrane. Along the outer border of the undulating membrane (UM) is the flagellum (F, Figs. 1, 3 and 6). The flagellum appears in cross section to be composed of 11 fibrils (Fig. 3), nine of which are peripherally located, whereas the other two occur as a pair in the center. Each fibril measures approximately 370 Å in diameter. Some of the fibrils are shown cut in longitudinal section in Figure 7.

The undulating membrane, in light microscope observations, takes a spiral course along the surface of the organism. Electron micrographs show that this organelle is in apposition to the body surface (UM, Figs. 1 and 6), having an internal structure composed of a homogeneous matrix of very low density limited by a membrane (UM, Fig. 3). The fibrils of the flagellum and matrix are all enclosed by a well differentiated outer covering (Fig. 3, UM).

Cytoplasm and Periplast. Most of the electron micrographs demonstrate that the cytoplasmic ground substance is finely granular. Scattered in the cytoplasm, without any definite spatial arrangement, are bodies which are interpreted as volutin granules (V, Figs. 1, 2 and 7). The rather large nucleus seems to be limited by a definite membrane (N, Fig. 1). The ground substance of the nucleus appears as a finely granular coagulum. In the center of the nucleus is an aggregation of dense granules, commonly called the endosome (E, Figs. 1 and 7).

The body of the organism is limited by the periplast, which sometimes gives the appearance of a double structure (P, Fig. 4). The periplast appears to be composed of a series of fine striations, in spiral arrangement (P, Figs. 2 and 3). The nature of these striations when the organism is cut parallel to its surface is shown in Figures 9 and 10. The striations are approximately 150 Å in thickness and spaced 105 Å apart.

DISCUSSION

Heretofore observations made with the aid of the electron microscope on species of the family TRYPANOSOMIDAE have been of whole mounted specimens, and observers have described the blepharoplast as a spherical body of which the internal structure could not be clearly discerned. It may be seen from the present electron micrographs that this organelle is limited by a membrane and also presumably marks the position where the flagellum originates. However, whether or not the fibrils of the flagellum originate from basal granules within the blepharoplast could not be determined.

The kinetoplast was also observed to be limited by a membrane with no demonstrable attachment to the blepharoplast. Cosgrove and Anderson (1954) demonstrated that after *Crithidia fasciculata* was subjected to desoxyribonuclease the ability of the kinetoplast to stain by the Feulgen technique was destroyed. It was also shown that the submicroscopic structure is essentially the same as that reported here for *T. equiperdum.*

Kleinschmidt and Kinder (1950) as a result of an electron microscope study of *T. lewisi* and *T. brucei* reported that the periplast consisted of a number of nearly parallel fibrils running longitudinally. Kraneveld, *et al* (1951) found a similar situation to exist in *T. evansi.* Lofgren (1950) reported essentially the same submicroscopic structure to exist in *Leishmania tropica* and stated that the periplast "appeared to be heavy fibrils contained in thin, elastic membrane." Das Gupta, *et al.* (1953), investigating *Leishmania donovani,* were of the opinion that their method of hydrolysing the organism with HCl, revealed ". . . longitudinal myoneme fibrils arranged in inter-connected bundles on the periplast." Meyer and Porter (1954) stated that the striations of *T. cruzi* were "just beneath the membrane or pellicle of the body." These authors suggested also that these striations were myonemes. It can be seen from the electron micrographs presented in this paper that the striations occur only on the surface membrane (pellicle). They appear to be the architectural pattern of the periplast and somewhat similar to that found in *Euglena* by Wolken and Palade (1953) and by Reger and Beams (1954).

No structure could be identified as typical mitochondria, i.e., having a structure similar to that demonstrated by other workers: Palade (1953), Beams and Tahmisian (1953), Sjöstrand (1953).

Structures that have been identified as volutin granules, are demonstrated scattered in the cytoplasm. It has been shown by van den Berge (1942, 1946) that after *T. gambiense* and *T. evansi* were subjected to ribonuclease, the volutin granules were removed. From this he concluded that the granules were actually a ribonucleoprotein. This has also been shown for *T. diemyctyli* by Barrow (1954). When cytochemical studies are completed on *T. equiperdum,* this may also be found to be true for this species.

SUMMARY

Ultra-thin sections of *Trypanosoma equiperdum* have been examined with the aid of the electron microscope. The blepharoplast appears to be limited by a membrane. In the center of the blepharoplastic area is the flagellum. The flagellum in cross-section is composed of nine peripheral and two central fibrils.

The kinetoplast is a rod-shaped structure limited by a membrane. Internally, this organelle consists of lamellae oriented at right angles to the long axis of the rod.

The undulating membrane is composed internally of a homogeneous matrix. This organelle is limited by a membrane which encloses the fibrils of the flagellum running along its outer border.

References Cited

Barrow, J. H. Jr. 1954 The biology of *Trypanosoma diemyctyli* (Tobey) II. Cytology and morphology of *Trypanosoma diemyctyli* in the vertebrate host, *Triturus v. viridescens*. Amer. Micr. Soc. **73:** 242–257.

Beams, H. W. and Tahmisian, T. N. 1953 Structure of the mitochondria in the male germ cells of *Helix* as revealed by the electron microscope. Anat. Rec. **117:** 610–611.

Cosgrove, W. B. and Anderson, E. 1954 The kinetoplast of *Crithidia fasiculata*. Anat. Rec. **120:** 813.

Das Gupta, N. N., Guha, A., and De, M. L. 1953 Observations on the structure of *Leishmania donovani*—the Kala-azar parasite. Exp. Cell Res. **6:** 353–360.

Kleinschmidt, A. and Kinder, E. 1950 Electronenoptische Befunde an Rattentrypanosomen. Zentralbl. Bakt., 1 Abt. **156:** 219–224.

Kraneveld, F. C., Houwink, A. L., and Keidel, H. J. W. 1951 Electron microscopical investigations on trypanosomes. I. Some preliminary data regarding the structure of *Trypanosoma evansi*. Proc. K. Akad. Wetensch. Amst. I. C. Biol. and Med. Sci. **54:** 393–399.

Lofgren, R. 1950 The structure of *Leishmania tropica* as revealed by phase and electron microscopy. J. Bact. **60:** 617–625.

Meyer, H. and Porter, K. R. 1954 A study of *Trypanosoma cruzi* with the electron microscope. Parasitology **44:** 16–23.

Palade, G. E. 1952 A study of fixation for electron microscopy. J. Exp. Med. **95:** 285–298.

———1953 An electron microscope study of the mitochondrial structure. J. Histochem. and Cytochem. **1:** 188–211.

Reger, J. F. and Beams, H. W. 1954 Electron micrographs of the pellicle of a species of Euglena. Proc. of Iowa Acad. of Sci. **61:** 593–597.

Sjöstrand, F. S. 1953 Electron microscopy of mitochondrial and cytoplasmic double membranes. Nature **171:** 30–32.

van den Berge, L. 1942 Sur la presence d'acide pentose-nucléique sous forme de grains de "volutine" dans le trypanosomes. Acta. Biol. Belg. **4:** 464–467.

———1946 A cytochemical study of the "volutin granules" in protozoa. J. Parasit. **32:** 465–466.

Wolken, J. J. and Palade, G. E. 1953 An electron microscope study of two flagellates. Chloroplast structure and variation. Ann. of N. Y. Acad. of Sci. **56:** 873–889.

Description of Plates

All electron micrographs show thin sections of *Trypanosoma equiperdum*. A magnification scale representing one micron is drawn on each figure.

Abbreviations used: N nucleus; E endosome; B blepharoplast; F flagellum; K kinetoplast; V volutin granule; UM undulating membrane; P periplast.

Plate I

Figures 1 and 2. Nearly longitudinal sections showing nucleus N, endosome E, volutin granules V, striated periplast P, undulating membrane UM, and flagellum F.

Figure 3. Tangential section showing blepharoplast B, flagellum F, undulating membrane UM, and striated periplast P.

Figure 4. Tangential section showing flagellum F. Note the periplast P, appearing as a double structure.

Plate II

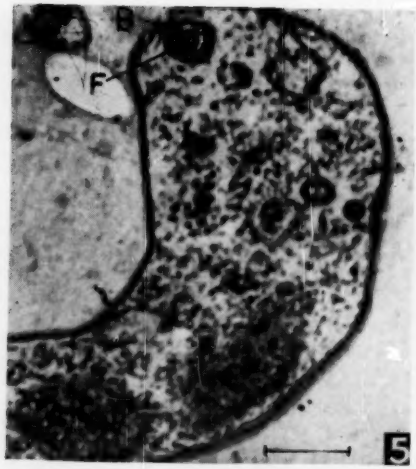
Figures 5 and 6. Tangential sections showing blepharoplast B, flagellum F, undulating membrane UM, and striated periplast P. Note kinetoplast K, with internal lamellae limited by a membrane.

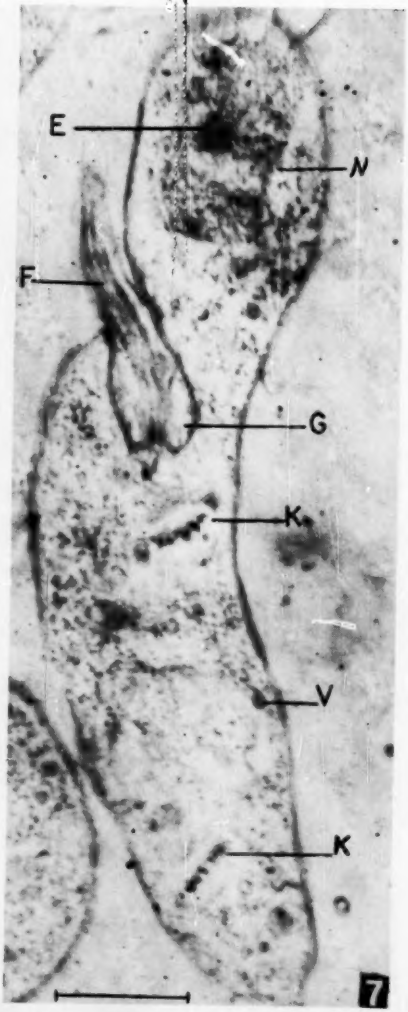
Figure 7. Tangential section showing two kinetoplasts K, nucleus N, and endosome E. Note longitudinal section of flagellum F.

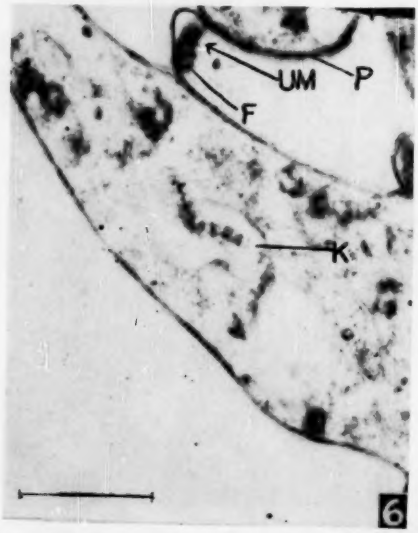
Figures 8, 9 and 10. Tangential sections showing flagellum F, kinetoplast K, and nucleus N. Note the striated periplast P, in Figure 10 cut parallel to the body surface.

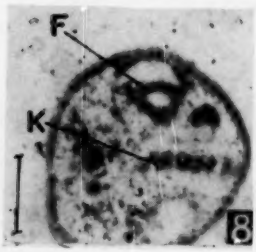
PLATE I

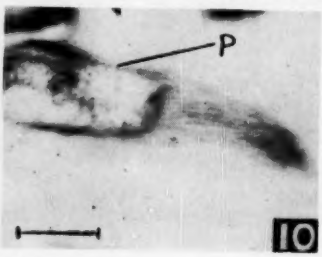
PLATE II











THE EFFECT OF HOST CASTRATION AND FASTING ON THE RATE OF GLYCOGENESIS IN *HYMENOLEPIS DIMINUTA**

JACK W. DAUGHERTY

The Rice Institute, Houston, Texas

Although a considerable amount of attention has been focused on the catabolic metabolism of carbohydrates in helminth parasites (Bueding and MacKinnon, 1955, and Read, 1951), little has been done to elucidate the mechanisms of carbohydrate synthesis. Glycogen has been shown to be of fundamental importance to the rat tapeworm, *Hymenolepis diminuta,* (Read, 1950; unpublished data). Therefore, a description of the process by which this substance is synthesized from the simple sugars available in the intestine of the host and a knowledge of the controls pertaining to this function are essential to a more complete understanding of the physiology and host-parasite relationship of this parasite. The present report contains the results of some early studies on the factors affecting glycogen synthesis in *H. diminuta.*

METHODS

Mature rats of the Sprague-Dawley strain were infected with two cysticercoids of *Hymenolepis diminuta* according to conventional methods (Addis and Chandler, 1944). Following the attainment of sexual maturity, the worms were harvested as needed, washed, and incubated in Krebs-Ringer-phosphate solution (38° C. pH 7.4) for 15 minutes. This was an attempt to equalize the glycogen levels of the individual worms. The worms were then washed in several changes of Krebs-Ringer-phosphate, blotted, weighed, and *ca.* 250 mg. portions of the anterior ends of separate worms were placed in chilled flasks containing 5 ml of an incubation medium adapted from Buchanan, Hastings, and Nesbett (1949): $CaCl_2$ (0.01M), KCl (0.65M), K-Pyruvate (0.04M) or Glucose (0.02M), $KHCO_3$ (0.008M), and $NaHCO_3$ (0.03M). Initial glycogen readings were obtained on worms withdrawn from the medium after 5 min. equilibration and final glycogen was determined following a subsequent incubation of duplicates for 60 minutes, in a Dubnoff incubator at 38° C. Glucose was also determined when the incubation medium contained pyruvate. For the glucose and glycogen determinations, the tissues were removed from the incubation flasks, dropped into 80% KOH, and were then placed in a boiling water bath. Following complete maceration the preparations were treated with an amount of ethanol suitable for the tissue involved (previously determined) and the glycogen centrifuged down. Glucose was determined on the supernatant. Glycogen was measured as glucose on the precipitate following H_2SO_4 hydrolysis. All glucose analyses were made according to the method of Folin-Malmros (1929), the readings being made on a Beckman Model B spectrophotometer. Worms from rats castrated two weeks prior to infection were also incubated as above and their activity compared with that of those from normal rats.

Received for publication, September 20, 1955.

* This investigation was supported in part by research grant E-374 from The National Institutes of Health, Public Health Service.

RESULTS AND DISCUSSION

Both the glucose and pyruvate fortified media supported glycogenesis to about an equal extent (see Table I). It is of interest to note that, although the glycogen content of the worms incubated in the pyruvate medium was increased, the glucose content was not significantly altered. This stability in glucose content confirms earlier results (unpublished) indicating that glucose in the tapeworm is handled in essentially the same fashion as in vertebrate tissues, where, as a potential metabolic intermediate (glucose-1-phosphate, glucose-6-phosphate), it is subject

TABLE I—*Effect of castration and food intake on carbohydrate synthesis in* H. diminuta

Exper. condit.	Run no.	Glucose *synthesis	Average	Glycogen **synthesis	Average
CONTROL					
Unfasted Glucose medium	1			+ 25.6	
	2			+ 18.2	
	3			+ 30.9	+ 20.5
	4	—	—	− 3.4	+ 7.6
	5			+ 31.6	
Fasted 24 Hr.	1			+ 40.2	
	2			+ 32.6	+ 36.7
	3	—	—	+ 37.4	± 5.4
Unfasted Pyruvate medium	1	− 0.53		+ 29.7	
	2	+ 0.35		+ 15.7	
	3	+ 0.35	+ 0.24	− 15.0	+ 15.0
	4	+ 1.10	± 0.28	+ 30.8	± 7.3
	5	− 0.98		+ 6.3	
	6	+ 0.09		+ 22.5	
Fasted 24 Hr.	1			+ 20.2	+ 20.0
	2			+ 16.3	± 5.0
	3	—	—	+ 23.4	
CASTRATE					
Unfasted Glucose medium	1			+ 2.0	
	2			− 0.9	
	3	—	—	− 10.1	− 2.6
	4			+ 4.8	± 2.9
	5			lost	
	6			− 8.9	
Fasted 24 Hr.	1			+ 5.3	+ 5.7
	2			+ 3.8	± 3.8
	3	—	—	+ 8.6	
Unfasted Pyruvate medium	1	+ 0.14		− 20.1	
	2	− 0.47		− 15.4	
	3	− 0.23	+ 0.07	− 25.6	− 14.7
	4	0.0	± 0.26	− 0.3	± 4.2
	5	+ 1.02		− 12.2	
Fasted 24 Hr.	1			− 6.8	− 9.7
	2			− 12.3	± 3.9
	3	—	—	− 10.0	

* Milligrams increase or decrease in glucose per gram of worm incubated.
** Milligrams increase or decrease in glycogen as glucose per gram of worm incubated.

to the principles that stabilize the concentration of any intermediate in a series of interdependent chemical reactions.

The data also indicate that castration of the rat host produced conditions that caused a decline in the rate of glycogen synthesis from both glucose (difference of castrate and normal means = 23.1 ± 8.1) and pyruvate (difference of means = 29.7 ± 8.4). This result appears in the table in the form of a decrease in the glycogen content of the worm after incubation. Since the glycogen content is a value dependent upon both the synthesis and utilization of glycogen, the value given is the net result of these two functions. There was no good evidence that a complete failure of synthesis was involved, although in those cases which showed a marked decline in glycogen it is possible that this condition was approximated.

The glucose content in the pyruvate medium, as before, was unaffected. It might be assumed from these data that an analysis of worm tissues from castrate hosts would show a reduced amount of glycogen (before incubation) when compared with that of worms from normal hosts. This has not been found. However, this is not unprecedented. A similar effect of host castration in a different area of tapeworm metabolism was demonstrated by Aldrich, Chandler, and Daugherty (1954). Although a marked decline in the activity of certain transaminase systems was observed, as in the present case, no effect was seen on the tissue concentration of the reactants. The meaning of these findings in terms of the effects of castration on the overall physiology of the tapeworm is not at present clear. However, it is possible that they are manifestations of a general reduction in the level of metabolism. The demonstrations of the wide-spread effects of host castration on the physiology of the tapeworm (lowered establishment, reduced transamination, increased fat deposition, and lowered carbohydrate synthesis) would seem to support this view. However, remaining mindful of the interdependent nature of the various aspects of intermediary metabolism, as found in those forms where information is more plentiful, the possibility that one or more key reactions are primarily involved cannot be ignored.

There were some indications that fasting the rat host for 24 hours prior to the experiment permitted a higher rate of glycogen synthesis from glucose. This did not seem to apply to the synthesis from pyruvate. Twenty-five hours of host starvation was shown earlier (unpublished) to be sufficient to reduce the glycogen stores in the anterior end of the worm by a considerable amount (from *ca.* 100 mgm./gm of worm to *ca.* 15 mgm./gm). It seems likely that the worm was able to respond to this loss by an acceleration in its rate of glycogenesis. Perhaps longer starvation would have caused a similar increase in the rate of synthesis from pyruvate. This effect of starvation is of fundamental interest in terms of the biochemical capabilities of tapeworms. It is apparent that, as has been pointed out elsewhere (Daughtery, 1955), they have not entirely lost the ancestral ability to regulate their internal physiological processes in response to environmental conditions.

The large variations in individual experimental results in the present work are, in part, attributable to the use of separate worms for each assay of glycogenesis, one for the zero reading, the other for the final glycogen assay. This was necessary as earlier results from this laboratory (unpublished) had shown a difference in the regional levels of tapeworm glycogen sufficient to preclude the use of different parts of the same worm for these determinations. In order to minimize the inherent error in the method, only double worm infections were used, with worms from the same rat being used for the zero time and final glycogen assays for each determination. However, even under these conditions it should be pointed out that differences between individual worms have been demonstrated, not only in regard to the content of intermediates but also in the rates of certain metabolic processes (Daugherty, 1955). Undoubtedly these differences are reflected in the present results.

Recently, attempts were made to demonstrate similar effects of castration on glycogen synthesis in the host's own tissues. These have been without success. On this basis it seems quite probable that the effects of castration on the physiology

of the rat tapeworm are produced through a secondary set of conditions arising in the body of the host and should not be attributed to any direct effect of the absence of the hormone on the parasite.

SUMMARY

A medium is given which supports glycogen synthesis from sodium pyruvate and glucose in the rat tapeworm. Castration of the host causes a decline in the rate of synthesis from both substrates. Host fasting accelerated the rate of synthesis from glucose but had no effect on the synthesis from pyruvate.

REFERENCES

ADDIS, C. J. AND CHANDLER, A. C. 1944 Studies on the vitamin requirements of tapeworms. J. Parasit. **30:** 229–236.

ALDRICH, D. V., CHANDLER, A. C., AND DAUGHERTY, J. W. 1954 Intermediary protein metabolism in helminths. II. Effect of host castration on amino acid metabolism in *Hymenolepis diminuta.* Exptl. Parasitology **3:** 173–184.

BUCHANAN, J. M., HASTINGS, A. B., AND NESBETT, F. B. 1949 The effect of the ionic environment on the synthesis of glycogen from glucose in rat liver slices. J. Biol. Chem. **180:** 435–446.

BUEDING, E. AND MACKINNON, J. A., 1955 Hexokinases of *Schistosoma mansoni.* J. Biol. Chem. **215:** 495–506.

DAUGHERTY, J. W. 1955 Intermediary Protein Metabolism in Helminths. III. The l-amino oxidases in *Hymenolepis diminuta* and some effects of changes in host physiology. Exptl. Parasitology. In press.

FOLIN, O. AND MALMROS, H. 1955 An improved form of Folin's micro-method for blood sugar determinations. J. Biol. Chem. **83:** 115–120.

READ, C. P. 1950 Rice Institute Dissertation. The Rice Institute Library.

————1951 Studies on the enzymes and intermediate products of carbohydrate degradation in the cestode, *H. diminuta.* Exptl. Parasitology **1:** 1–18.

RESEARCH NOTE

OPHIOTAENIA GRANDIS LA RUE (CESTODA: PROTEOCEPHALIDAE) IN McCURTAIN COUNTY, OKLAHOMA

During the summer of 1955 students at the University of Oklahoma Biological Station collected two cottonmouth water moccasin snakes, *Ancistrodon piscivorus* Lacépède. One host was taken near the Little River Bridge between Idabel and Broken Bow, the other in the Beavers Bend State Park, both in McCurtain County. The intestine of each snake contained approximately twenty *Ophiotaenia grandis* La Rue (1911, Zool. Anz. **38:** 473–482). La Rue described *O. grandis* from fragments taken from *A. piscivorus* from the New York Zoological Gardens. The site of capture of the snake was not known. I know of no record of this parasite other than the original description.

The longest of La Rue's specimens measured 200 mm. He estimated the total length to be from 300 to 400 mm. I found the worm very fragile; however, I was able to recover one individual which was 550 mm in length. Specimens have been deposited in the Parasitological Collection of the University of Oklahoma Museum.

The Beavers Bend State Park host was also infected with several much smaller tapeworms. Although failure to recover mature proglottids made positive identification impossible, various measurements and the position of the genital pore indicated that it was *Ophiotaenia agkistrodontis* Harwood (1933, Parasitology **25:** 130–142). I should like to express my appreciation to Dr. J. Teague Self, under whose supervision all work was carried out.—LARRY S. ROBERTS, *Southern Methodist University, Dallas, Texas.*

PROTEOLYTIC ACTIVITY IN EXTRACTS OF THE ESOPHAGUS OF ADULTS OF *ANCYLOSTOMA CANINUM* AND THE EFFECT OF IMMUNE SERUM ON THIS ACTIVITY*

RALPH E. THORSON

Department of Pathology and Parasitology, School of Veterinary Medicine
Alabama Polytechnic Institute, Auburn, Alabama

It has been suggested that protective immunity against nematode infections is an enzyme-antienzyme mechanism in which enzymes used by the worm for invasion and nutrition serve as antigens, and antibodies formed against them in the host inhibit their activity and create an unfavorable environment for the parasite (Chandler 1932, 1936, 1953; Thorson, 1953). The present study is concerned with proteolytic activity in extracts of the esophagus of adults of *Ancylostoma caninum*, the dog hookworm, and the effect of serum from dogs refractory to further infection upon this proteolytic activity.

MATERIALS AND METHODS

Adults of *A. caninum* were removed from the intestines of autopsied dogs. After several washings in physiological saline, the esophagus, cephalic glands (amphidial glands of Cobb) and cervical glands (excretory glands of Cobb) were dissected from the worms and placed in separate containers. In the present study only the esophagus was used. The esophagi in a small volume of saline (1 ml) were ground in a tissue grinder until there were no whole cells left. The ground tissue was placed for varying periods of time (several hours to overnight) in a refrigerator at 5° C. After this, the preparations were centrifuged to remove the cellular debris. An aliquot of the supernatant was then analyzed for protein nitrogen using ultramicro Kjeldahl diffusion cells (Kirk, 1950). Experimental results are expressed as amount of activity per microgram of protein nitrogen in the esophagus extract.

A 3 per cent solution of casein was used as a substrate for determining the presence of proteases in the extracts of the esophagus. It was prepared by suspending 3 grams of casein in some distilled water in a 100 ml volumetric flask and adding 36 ml. 0.1 normal sodium hydroxide slowly while mixing. The mixture was heated at 30° C. for 30 minutes in a water bath to get the casein in solution. The preparation was then brought to volume with distilled water.

Since the volumes of reactants were small, special microliter pipettes and pipette controls (Microchemical Specialties Co., Berkeley, California) were used. A capillary burette with a total volume of 50 microliters was used in all titrations. A glass thread magnetic stirrer was inserted into the mixtures during titrations.

To assay proteolytic activity, a microliter modification of Sorensen's formol titration was used (Kirk, 1950). Fifty microliters of the casein solution were placed in a tube with 25 microliters of esophagus extract. Two-tenths molar phos-

Received for publication, October 3, 1955.

* Approved by the Committee on Publications, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama, Publication No. 526.

This investigation was supported in part by research grant E872 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

phate buffers in the amount of 25 microliters were used in enzyme reactions when the pH optimum was determined, and thereafter in all reactions buffer at pH 6 was added to each preparation. Controls of boiled extract and casein, of casein, and of extract were prepared for each observation. The mixtures were incubated at 37° C. for varying periods of time, after which ten microliters of a 0.1 per cent alcoholic phenolphthalein solution were added to each preparation. The preparations were then neutralized to the phenolphthalein endpoint with 0.02 N sodium hydroxide. One hundred microliters of a formaldehyde solution (1 volume of 37 per cent formaldehyde: 2 volumes of distilled water) were added and titration continued to the first appearance of pink color. The titrations were performed quickly to avoid the possibility of carbon dioxide absorption and consequent fading of the color at the endpoint. (Titrations in a carbon dioxide free atmosphere did not differ significantly from those in air if the titrations were done rapidly.)

EXPERIMENTAL RESULTS

Extracts of the esophagus of adults of *A. caninum* were reacted with casein for varying periods of time to determine if the esophageal glands contained substances with proteolytic activity. A typical protocol is shown in Table 1. There

TABLE 1.—*Determination of proteolytic activity using extracts of esophagus of* Ancylostoma caninum *and casein*

	Tube Number									
	1	2	3	4	5	6	7	8	9	10
Casein (3%), microliters	50	50	50	50	50	50	50	50	50	—
Phosphate buffer, pH 6, microliters	25	25	25	25	25	25	25	25	25	25
Saline (0.85%), microliters	—	—	—	—	—	—	—	—	25	50
Esophagus extract, microliters	25	25*	25	25*	25	25*	25	25*	—	25
Reaction time in hours	0		1		2		4		0	
Microliters of 0.02 N NaOH to phenolphthalein endpoints**	7.7	7.9	10.9	8.3	17.2	8.5	17.3	8.5	5.8	2.1
Microliters of 0.02 N amino acids	0		2.6		8.7		8.8			
Microliters of 0.02 N amino acid per microgram protein nitrogen of extract	0		5.6		18.4		18.5			

* Extract boiled in a water bath for 10 minutes at 100° C.
** After neutralization and addition of 100 microliters of formaldehyde solution.

was pronounced proteolytic activity in all extracts of esophagus which were tested.

Since the activity of enzymes varies with hydrogen ion concentrations, determinations of activity were made with phosphate buffers at a pH of 5.3, 6.0, 7.0, and 8.0. The preparations were identical to those in Table 1 except that the pH of the buffer was varied. The preparations were incubated at 37° C. for two hours, the time required for maximum activity, and were then titrated. This experiment indicated that the proteolytic activity was greatest at a pH of approximately 6 (Table 2).

TABLE 2.—*The effect of hydrogen ion concentration on proteolytic activity in esophageal extracts of* Ancylostoma caninum

pH	Microliters of 0.02 N amino acids produced per microgram of protein nitrogen of extract
5.3	6.0
6.0	23.5
7.0	5.7
8.0	2.0

All preparations were incubated at 37° C. for two hours.

Since there was proteolytic activity in extracts of the esophagus of *A. caninum*, it was of interest to determine if this activity was inhibited by serum from dogs refractory to further infection with hookworm. This serum, hereafter referred to as immune serum, was obtained from a dog which had been infected at approximately monthly intervals during a four-year period. The number of larvae given to this dog was gradually increased until it withstood effectively as many as 80,000 to 100,000 infective larvae. Serum from young puppies with a minimum of experience with hookworms was used as a control and will be referred to as "normal serum."

Twenty-five microliters of esophagus extract were placed in each of 6 tubes. To two of the tubes 25 microliters of saline were added, to two others 25 microliters of normal serum were added, and to the last two 25 microliters of immune serum were added. After a 15-minute reaction period buffer (25 microliters) and casein (50 microliters) were added to each tube. One of the tubes with esophagus extract and saline, one with extract and normal serum, and one with extract and immune serum were titrated immediately after adding the casein and buffer. The other tubes were placed in an incubator at 37° C. for two hours. Appropriate controls were also prepared and titrated. After incubation, the last three tubes were titrated. Since the three tubes which had been titrated immediately (0 time) served as controls, their titration values were subtracted from the corresponding figure for a tube with a 2-hour incubation period. A summary of the results is shown in Table 3. There was an appreciable inhibition of the proteolytic activity in esophagus

TABLE 3.—*Inhibition of proteolytic activity of extracts of esophagus of* A. caninum *with serum from a dog refractory to further infection with the worm*

	Microliters of 0.02 N amino acids produced per microgram of nitrogen in esophagus extract during a 2 hour reaction period
Esophagus extract + saline + casein + buffer	15.21
Esophagus extract + normal serum + casein + buffer	14.75
Esophagus extract + immune serum + casein + buffer	1.70

extracts of *A. caninum* when serum from a dog refractory to further infection was added to the extract before the substrate casein was added. If either of the controls, extract with saline or extract with normal serum is compared with the extract and immune serum, the degree of inhibition is seen to be about 88 per cent.

DISCUSSION

The presence of proteolytic enzymes in nematodes has been based for the most part on histological evidence (see Von Brand, 1953, for review). There have been studies of proteolytic enzymes in the intestines of nematodes by Abderhalden (1911), Flury (1912), Rogers (1941) and Carpenter (1952). Lewert and Lee (1954) have indicated that the infective larvae of *A. caninum* possess an enzyme or enzyme complex which is active against gelatin. Chitwood (1938) and Carpenter (1952) have given good chemical evidence for proteolytic activity in the esophagus of *Ascaris*. This study demonstrates that there is proteolytic activity in the extractions of the esophagus of adults of *A. caninum*. Presumably, these extractions contain the secretions of the esophageal glands. Anatomically, the esophageal glands open between the dorsal teeth in the buccal cavity and not into the lumen of the esophagus. Furthermore there is histological evidence that extracorporeal

digestion is used by nematodes for the purpose of obtaining food. This leads to the supposition that the proteolytic activity described herein may play a role in extracorporeal digestion.

The pH optimum of this activity was approximately 6 which would indicate that the activity is not of host origin, since the optimum activity of intestinal enzymes of dogs is in the alkaline range.

That serum from a dog refractory to further infection with *A. caninum* would inhibit this proteolytic activity was suspected and confirmed. The inhibition by immune serum and not by normal serum of dogs indicates that while worms fed in the intestine, substances containing this activity were released and served as antigens. Whatever the function of this activity, presumably feeding, the inhibition of it by immune serum gives a very direct clue to the mechanism of immunity in nematode infections. Chandler (1932, 1936) first presented the hypothesis that antibodies in nematode infections acted as specific antienzymes which "prevent the worms from digesting and assimilating the host's proteins." The writer (1953) as a result of a study with *Nippostrongylus muris* from the rat presented a similar hypothesis which stated that "antibodies produced by the host against the enzymes in the secretions and excretions of the worms would react against the enzymes of the invading larvae to slow down their migration and by adversely affecting their nutrition would interfere with their normal development. This would allow the host's reticulo-endothelial system to attack and encapsulate many of the larvae in the tissues and would prevent the worms that reached the intestine from establishing normal infections." The present study gives additional evidence for this hypothesis, since antibodies are formed in the dog against the enzyme(s) responsible for proteolytic activity in extracts of the esophagus of the hookworm, *A. caninum*, and these antibodies are efficient inhibitors of this activity. Inhibition by antibodies of proteolytic activity which is essential to the physiology of the hookworm would create a very unfavorable environment for the worm in the intestine. The feeding habits of the worm, particularly blood sucking, create an unusually favorable contact between the proteolytic enzymes of the esophageal glands which may serve as antigens and the antibodies acting as antienzymes of the blood.

It should be stated that the foregoing discussion does not indicate that the enzyme-antienzyme hypothesis is restricted to just one enzyme or group of enzymes in the esophagus but rather it should be emphasized that the enzyme-antienzyme (antigen-antibody) relationship is very complex in helminthic infections and that good protective immunity may consist of a mosaic of such reactions.

SUMMARY

1. Extracts of the esophagus of adults of *Ancylostoma caninum* showed proteolytic activity when casein was used as a substrate at a pH optimum of approximately 6.0.

2. Serum from a dog refractory to further infection with *A. caninum* inhibited this proteolytic activity by as much as 88 per cent.

REFERENCES CITED

ABDERHALDEN, E. 1911 Ueber den Gehalt von Eingeweidewurmen an peptolytischen Fermenten Z'tschr. Physiol. Chem. **74**: 409–410.

VON BRAND, T. 1952 Chemical physiology of endoparasitic animals. Academic Press, Inc., New York, New York.

CARPENTER, M. F. P. 1952 The digestive enzymes of *Ascaris lumbricoides* var. *suis:* their properties and distribution in the alimentary canal. Doctoral dissertation, University of Michigan. Available from University Microfilms, Ann Arbor, Michigan.

CHANDLER, A. C. 1932 Susceptibility and resistance to helminthic infections. J. Parasit. **18:** 135–152.

————1936 Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in *Nippostrongylus* after transfer from immune to non-immune rats. Am. J. Hyg. **23:** 46–54.

————1953 Immunity in parasitic diseases. J. Egypt. Med. Asso. **36:** 811–834.

CHITWOOD, B. G. 1938 Notes on the physiology of *Ascaris lumbricoides.* Proc. Helminthol. Soc. Washington **5:** 18–19.

FLURY, F. 1912 Zur chemie und toxocologie der Ascariden. Arch. exper. Path. u. Pharmakol. **67:** 275–392.

KIRK, P. L. 1950 Quantitative Ultramicro Analysis. John Wiley and Sons, Inc., New York, New York.

LEWERT, R. M. AND LEE, C. 1954 Studies on the passage of helminth larvae through host tissues. II. Enzymatic activity of larvae *in vitro* and *in vivo.* J. Inf. Dis. **95:** 35–51.

ROGERS, W. P. 1941 Digestion in parasitic nematodes. III. The digestion of proteins. J. Helminthol. **19:** 47–58.

THORSON, R. E. 1953 Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris.* Am. J. Hyg. **58:** 1–15.

RESEARCH NOTE

SOME HELMINTHS OF OREGON URODELES

During the spring of 1954, some salamanders from the Willamette Valley, Oregon, were examined for helminths; the findings here reported are in addition to those presented earlier by the writer (1954, J. Parasit. **4:** 231). The animals examined included: 17 larval *Ambystoma gracile* from Linn Co., 19 *Plethodon dunni,* 26 *P. vehiculum* and a single *Rhyacotriton olympicus,* all from Benton Co. Helminths were found in 3 of the 4 species, *P. vehiculum* being uninfected, and all worms recovered constitute new host records.

The parasites and their hosts are:

TREMATODA:

Megalodiscus americanus Chandler 1923, from the rectum of all of the larval *Ambystoma gracile.*

Monostome cercariae found encysted upon the rectal mesentery of a single *A. gracile.*

NEMATODA:

Hedruris siredonis Baird 1858, were found in the stomach of 6 *A. gracile.*

Oxyuris dubia Leidy 1856, from the rectum of the single *Rhyacotriton olympicus* and the same location in 3 *Plethodon dunni.*—DONALD L. LEHMANN, *Department of Zoology, College of the Pacific, Stockton, Calif.*

THE EFFECT OF EXTRACTS OF THE AMPHIDIAL GLANDS, EXCRETORY GLANDS, AND ESOPHAGUS OF ADULTS OF *ANCYLOSTOMA CANINUM* ON THE COAGULATION OF DOG'S BLOOD*

R. E. THORSON

Department of Pathology and Parasitology, School of Veterinary Medicine
Alabama Polytechnic Institute, Auburn, Alabama

Substances that inhibit the coagulation of blood have been found in several species of nematodes (Loeb and Smith, 1904, 1904a; Loeb & Fleisher, 1910; Weinberg, 1907; Schwartz, 1921; Hoeppli and Feng, 1933). Loeb and coworkers determined that extracts of *Ancylostoma caninum* in physiological saline inhibit the coagulation of dog's blood *in vitro* for periods which vary with different samples of blood, the maximum delay in coagulation observed being about 24 hours. This study was undertaken to determine the mechanism of this inhibition of coagulation and to determine the effect that immune serum would have on the inhibition.

MATERIALS AND METHODS

Adults of *Ancylostoma caninum* were removed from dogs' intestines at autopsy. After the worms were washed several times in physiological saline, the esophagus, the amphidial glands, and the excretory glands were dissected from them. The isolated portions of about 100 worms were placed in separate containers, ground with 2 ml of saline and allowed to extract for varying periods (several hours to overnight) at 5° C. The supernatants designated as amphidial extract, excretory extract, and esophagus extract were then used in blood clotting tests.

In an isolated thrombin-fibrinogen clot reaction, thrombin from a commercial source was used. Plasma was separated from blood drawn from dogs, and the fibrinogen was precipitated with a saturated sodium chloride solution (equal volumes of saturated saline and plasma). After centrifugation, the supernatant was removed, and the precipitate containing fibrinogen was resuspended in physiological saline (0.85 per cent). The fibrinogen was precipitated again, resuspended in saline and used in tests with thrombin. A unit of thrombin has the power to clot 1.0 ml of fibrinogen solution in 15 ± 0.5 seconds at 28° C.; so the fibrinogen was formulated so that one unit of thrombin would clot it under the above conditions.

For prothrombin time determinations a commercial thromboplastin[1] and dog plasma were used. The thromboplastin was prepared according to the manufacturer's description. Blood was drawn from a dog and added to an amount of 0.1 molar solution of sodium oxalate such that the final concentration of the oxalate was 0.01 molar. The blood was centrifuged to remove the cellular elements, and the plasma was withdrawn and used immediately. Two-tenths ml of thromboplastin

Received for publication, October 3, 1955.

* Approved by the Committee on Publications, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama, Publication No. 527.

This investigation was supported in part by research grant E 872 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

[1] The author wishes to express his appreciation to the Warner-Chilcott Laboratories, New York, who supplied the "Simplastin" used in this study.

were pipetted into a test tube and the tube placed in a water bath at 37° C. After several minutes 0.1 ml of plasma also at 37° C. was added. On addition of plasma to the thromboplastin, a stopwatch was started. A nichrome wire loop was inserted and moved across the bottom of the tube with sweeping motions until a clot appeared. The time was noted and this was taken as the normal prothrombin time for the plasma sample. When extracts were placed with the thromboplastin, saline in equivalent volumes was added to other tubes as controls. Six determinations were made for each experimental manipulation and the standard error rarely exceeded 0.2 seconds.

Serum from a dog which had been infected with larvae of *A. caninum* periodically for 4 years is designated as immune serum, and normal serum was obtained from young dogs that had had little experience with hookworms.

EXPERIMENTAL RESULTS

A. Effect of amphidial gland, excretory gland, or esophagus extracts on thrombin-fibrinogen reaction.

Since Loeb and co-workers had determined that the substance responsible for inhibition of coagulation was present in the anterior half of the worms, it was felt that the extractions of either the amphidial glands, excretory glands, or esophagus might contain the active substances. In addition, if this activity was present in any of these extracts, it was of interest to determine if the activity was directed against the thrombin-fibrinogen stage of the coagulation reaction.

One ml of fibrinogen solution was added to a tube containing 0.5 ml of extract plus one unit of thrombin. The determinations were carried out in triplicate. Instead of extract, saline was added to similarly prepared tubes as controls. The normal clot time was 15 ± 0.5 seconds, and in no instance did the extracts of amphidial glands, excretory glands, or esophagus increase this time. It was concluded that the extracts of esophagus, amphidial glands, and excretory glands did not contain the anticoagulant described by Loeb and coworkers or that the activity, if present, was not directed at the thrombin-fibrinogen stage of the coagulation reaction of blood.

B. Effect of amphidial gland, excretory gland, and esophagus extracts on prothrombin time of dogs' plasma.

Since there was no delay in coagulation when a thrombin-fibrinogen clotting system was used, a prothrombin time determination with these extracts added was used. Fifty microliters of one of the extracts were added to 0.2 ml thromboplastin before 0.1 ml of plasma was added to the reaction. Saline was added in place of the extracts for controls. A summary of the results is given in Table 1. It can

TABLE 1.—*Effect of extracts of amphidial glands, excretory glands, and esophagus of* Ancylostoma caninum *on prothrombin time of dogs' plasma.*

	Time in seconds, mean of 6 determinations
Normal prothrombin time	7.5
Prothrombin time with 0.05 ml saline added	7.7
Prothrombin time with 0.05 ml esophagus extract added	8.3
Prothrombin time with 0.05 ml excretory gland extract added	7.7
Prothrombin time with 0.05 ml amphidial gland extract added	16.6*

* A complete clot never formed.

be seen that the only delay of prothrombin time occurred when an extract of the amphidial gland was mixed with thromboplastin before plasma was added. In addition, these tubes had very small clots, while the preparations in all of the other tubes were completely clotted.

A portion of the amphidial gland extract was boiled in a water bath for 15 minutes to determine if the activity was heat stable. The boiling did not destroy the activity of the extract as the prothrombin time remained at 10 seconds and the clot was still incomplete.

C. Effect of immune serum and normal serum on the ability of extracts of amphidial glands to increase the prothrombin time of dogs' plasma.

Because there was delay of the prothrombin time of dogs' blood using an extract of amphidial glands from *A. caninum*, it was suspected that serum from a dog refractory to further infection with *A. caninum* might inhibit this activity. Before being placed with thromboplastin the amphidial extract was mixed with 25 microliters of physiological saline, normal serum or immune serum. After 15 minutes, thromboplastin was added and the tests were performed as before. The results are summarized in Table 2. Both normal serum and immune serum reduced the

TABLE 2.—*The effect of saline, normal serum and immune serum on the ability of the amphidial extract of adults of* Ancylostoma caninum *to delay the prothrombin time of dogs' plasma.*

	Time in seconds, mean of 6 determinations
Prothrombin time with 0.075 ml saline added	7.9
Prothrombin time with 0.025 ml saline and 0.05 ml amphidial gland extract added	15.0*
Prothrombin time with 0.025 ml normal serum and 0.05 amphidial gland extract added	11.1*
Prothrombin time with 0.025 ml immune serum and 0.05 ml amphidial extract added	11.8*

* A complete clot never formed.

delay of the prothrombin time somewhat, but as in the previous experiment a complete clot never formed in any of the tubes except those to which only saline had been added. Since immune serum did not reduce the delay of the prothrombin time any more than normal serum and since the clot formed was still incomplete, it must be concluded that this immune serum does not inhibit the anticoagulant activity of extracts of amphidial glands.

DISCUSSION

Substances from the dog hookworm, *Ancylostoma caninum*, are capable of inhibiting the coagulation of the blood of dogs as has been demonstrated by Loeb and Smith (1904, 1904a), Loeb and Fleisher (1910) and this paper. The purpose of the present study was to eliminate to some extent the variability of results obtained when whole blood was used in the clot formation. Also, it was desirable to determine from which portion of the worm this activity was derived.

The inhibition of coagulation seems to be present in the amphidial glands and is directed against the formation of thrombin from prothrombin. It was not possible to determine by this study whether the amphidial gland extract acted directly on the prothrombin or the thromboplastin. Loeb and Smith's observation that the activity was heat stable is corroborated by this study. Since heat does not destroy

the activity, it might be assumed that the active substance is not a protein, since it would be denatured by heat. Denaturation does not imply loss of activity in proteins but it suggests strongly that such is the case.

Since other studies (Thorson, 1953, 1956) have shown that immune serum inhibits activities of enzymes which may be found in worms, it was felt that this substance which delays blood coagulation might be inhibited by immune serum as well. The prothrombin time of plasma mixed with amphidial gland extract and thromboplastin was reduced somewhat when either normal or immune serum were mixed with the extract prior to adding the other ingredients, but the clot in either case was much smaller than that in tubes in which only saline, thromboplastin, and plasma were used. The substance which prevents coagulation does not seem to act as an antigen in infections with *A. caninum* in dogs, since it was not inhibited by a serum from a dog refractory to further infection with this parasite anymore than by normal serum. However, the anti-coagulant may be a complex molecule composed of proteins, lipids, and polysaccharides; and antibodies may be formed to parts of the molecule which do not play a role in the delay of clot formation. On the other hand, the substance may be a very simple compound which is not antigenic in the dog. Further characterization of the chemical nature of the anticoagulant may lead to clarification of this point.

The fact that bleeding continues at the site of attachment by adult hookworms after the worms leave is the only evidence we have that they secrete an anticoagulant. Morphologically, there are ducts from the amphidial glands to the edges of the lips, but the present study does not prove that this anticoagulant is secreted by the worm. However, before the present study was initiated, it was determined that if adults of *A. caninum* were "milked" by rolling a bent glass rod over the anterior end, substances were released which did delay somewhat the coagulation of freshly drawn blood. This is at least an indication that the anticoagulant may be secreted by the hookworm.

SUMMARY

1. Physiological saline extracts of the esophagus, excretory glands, or amphidial glands of adults of *Ancylostoma caninum* do not delay coagulation when thrombin and fibrinogen are used in the clot reaction.

2. Physiological saline extracts of the amphidial glands of *A. caninum* increase the prothrombin time of dog plasma, and, in addition, the clot formed in these reactions is not complete when compared to those formed when saline, extracts of esophagus, or extracts of excretory glands are used instead of the amphidial gland extract.

3. Both normal and immune serum reduce somewhat the delay in prothrombin time caused by amphidial gland extracts but the clot formed is still small when compared to controls where saline is used instead of amphidial gland extracts.

REFERENCES CITED

HOEPPLI, R. AND FENG, L. C. 1933 The presence of an anticoagulin in the esophagus of *Bunostomum trigonocephalum* from the intestine of sheep. Arch. Schiffs—u. Tropen-Hyg. **37:** 176–182.

LOEB, L. AND FLEISHER, M. S. 1910 The influence of extracts of *Anchylostoma caninum* on the coagulation of the blood and on hemolysis. J. Inf. Dis. **7:** 625–631.

——— AND SMITH, A. J. 1904 The presence of a substance inhibiting the coagulation of blood in *Anchylostoma*. Proc. Path. Soc. Phila., n. s. **7**: 173–178.

——— AND ———1904a Ueber eine die Blutgerinnung hemmende Substanz in *Anchylostoma caninum*. Centralbl. Bakteriol., 1. Abt. Orig. **37**: 93–98.

SCHWARTZ, B. 1921 Effect of secretions of certain parasitic nematodes on coagulation of blood. J. Parasit. **7**: 144–150.

THORSON, R. E. 1953 Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris*. Am. J. Hyg. **58**: 1–15.

THORSON, R. E. 1956 Proteolytic activity in extracts of the esophagus of adults of *Ancylostoma caninum* and the effect of immune serum on this activity. J. Parasit. **42**: 21–25.

WEINBERG, M. 1907 Sur une hemotoxine d'origine vermineuse. Compt. rend. soc. biol **63**: 13–15.

RESEARCH NOTE

NOTES ON THE USE OF TECHNICON MOUNTING MEDIUM FOR IN TOTO PREPARATIONS

Refractile structures, such as cilia, spines, and setae, are often obscure in conventional mounting media, in which they become excessively clear and easy to overlook, due to the small difference in refractive index between the structure and the medium. This is particularly true of specimens which are described from preserved collections and have never been observed in life or in an aqueous medium, conditions under which such structures are most easily seen.

In recent studies in this laboratory, ciliates, trematodes, and cestodes have been prepared as whole mounts in Technicon synthetic medium, having a very high refractive index (1.65), as well as gum damar (R.I. 1.52) and Clarite (R.I. 1.54). Methyl salicylate was used as a clearing agent. The size and distribution of cilia on *Haptophrya michiganensis* (Ciliatea) were most clear in Technicon mounts. Among the trematodes studied, cuticular spines were seen in greatest detail in specimens of *Acanthatrium* spp., *Prosthodendrium* sp., *Myotitrema* sp., *Acanthostomum* spp. and *Urotrema* spp. mounted in Technicon medium. The characteristic atrial spines in specimens of *Acanthatrium* were particularly easy to study. Specimens of *Plagitura salamandra* and *P. parva* show their cuticular spines clearly and equally well in Technicon mounts. It is of interest to note that *P. salamandra* was originally described as lacking cuticular spines (Holl, F. J., 1928, J. Elisha Mitchell Sci. Soc. **43**: 181–186) and later shown to possess them (Kelley, R. S., 1934, J. Parasit. **20**: 324–325). Observations on a number of unidentified cestodes have indicated equally good results with respect to the hooks and spines on the scolices.

Investigations on the efficacy of Technicon medium with respect to cercarial stylets, spines, and setae are being made as material becomes available. Previous work on species of xiphidiocercariae (Etges, F. J., 1953, J. Parasit. **39**: 643–662.) was almost limited to living specimens, since their stylets and cuticular spines were not visible in Clarite and gum damar mounts. This difficulty limits greatly the value of type specimens of such cercariae as toto mounts because such characteristics are diagnostic. It is hoped that this limitation may be overcome by using a medium with a very high index of refraction.

No fading or acidity has been noted in specimens stained with carmine or hematoxylin, mounted in Technicon, and placed in daylight for more than six months. The medium itself shows some evidence of yellowing at the edge of some slides, but no cracking or crumbling has been noted.—FRANK J. ETGES, *Department of Zoology, University of Cincinnati.*

OBSERVATIONS ON THE PLEROCERCOID LARVA OF *PELICHNIBOTHRIUM SPECIOSUM* MONTICELLI 1889

NATHAN W. RISER

Biology Department, Fisk University, Nashville, Tenn.

On February 6, 1947, Mr. J. B. Philips of the California State Fish and Game Commission took a specimen of *Alepisaurus ferox* Lowe which had been entangled in the algae off Carmel, California on February 4th. On autopsy, 20 large plerocercoid larvae of *Pelichnibothrium speciosum* Monticelli, 1889 were found in the intestine and blind gut. In addition, several hundred small specimens (*cir.* 20 mm. in length) which fit the description of *Pelichnibothrium caudatum* Zschokke and Heitz, 1914 and were very similar to *Phyllobothrium* sp. (Fig. 556) of Joyeux and Baer (1936) were recovered. (Similar larvae were present in the cecum of the cephalopods *Dosidicus gigas* (d'Orbigny) from Corona del Mar, California, and *Loligo opalescens* Berry from Monterey Bay, California.) Tiny plerocercoids (0.4 mm. long) which had much the same appearance as the small ones just mentioned were present in the blind gut of *A. ferox* by the thousands, giving the chyle the appearance of being filled with sand. No intermediate sizes which would link the three larvae were present. The musculature of the host (*A. ferox*) contained innumerable plerocercoids of *Tentacularia coryphaenae* Bosc, 1802. Additional specimens of the host from Monterey Bay, came under observation in 1947 (March 26, June 9, July 7, July 12). These five were the only specimens of this relatively rare fish which were encountered in the area between January 1, 1946 and September 1, 1949.

Over 70 of the large specimens of *P. speciosum* were recovered from the five fish. The largest was 1450 mm. in length with a tail constituting 640 mm. of the length, and the smallest was 265 mm. long, the tail being 142 mm. of that length. The worms were of interest because they were plerocercoids with larval tails into which they could invaginate, and yet, they were strobilate, and the proglottids contained complete sets of genitalia. A descriptive diagnosis of the larvae follows; all measurements in millimeters.

Pelichnibothrium speciosum, plerocercoid larva

Strobilate, acraspedote plerocercoids reaching length of over 1400 and consisting of about 200 proglottids with posterior appendages constituting 120–640 of total length. Scolex large, bearing four large phyllidia each with an anterior acetabulum 0.33–0.36 in diameter. Apical sucker functional, 0.225–0.285 in diameter. Strobila beginning immediately behind scolex. First proglottids broader than long, posterior proglottids square, 3 by 3. Cuticle thin. External longitudinal muscles very numerous in mid-line. Internal longitudinal muscle layer consisting of about 40 bundles of fibers in center of proglottid; forming diamond-shaped ring around inversion tube; ventral bundles small and weak. Longitudinal muscle bundles in caudal appendage widely separated and strongly developed (Fig. 1). Dorsal excretory vessels 0.027–0.033 in diameter; ventral vessels 0.027–0.090. Testes, 300–400, two to three layers deep in two lateral fields in each proglottid, 0.075–0.090 by 0.12–0.15 in diameter. Genital atria marginal, irregularly alternating in posterior third of proglottid. Cirrus pouch 0.66–0.73 long by 0.19–0.25. Cirrus armed with small spines with long filamentous ends. Vagina rising anteriorly from genital pore, turning posteriad in mid-line at top of uterus; lumen lined with very long cilia. Uterine duct opening into uterus at level of genital atrium. Ovary 0.45–0.55 long by 0.54. Vitellaria enclosing testicular fields, extending anterior of fields and behind ovary.

Monticelli (1889) in his original description of larvae from *Alepisaurus* made no mention of the internal anatomy. The larvae described by Zschokke and Heitz (1914)

Received for publication, June 6, 1955.

as *P. caudatum* as well as the "*Pelichnibothrium* larvae" of Yamaguti (1934) were not strobilate, and possibly, when our understanding of phyllobothriid life-histories advances, may prove to be plerocercoids of a species of *Phyllobothrium*. Yamaguti (1934) described what he considered to be adult specimens of *P. speciosum* from *Prionace glauca*. The primary differences between his description and the one given above, center around the much greater size of the specimens from *Alepisaurus* and the correspondingly larger size of the organs. One difference is difficult to reconcile. Yamaguti stated that the larval tail "detached when about twenty" proglottids had formed, but in the present material, the first twenty proglottids containing genitalia occurred in the initial twenty millimeters of the strobila, and were followed by over 150 proglottids of which the last 30 to 50 contained a fully developed uterus. Yamaguti mentioned the testes as numbering approximately 200. The actual number is difficult to determine since the testes are in more than one layer, and are completely enclosed by the vitellaria. Proglottids which had the vitellaria dissected away showed 325 to 387 testes in the California specimens.

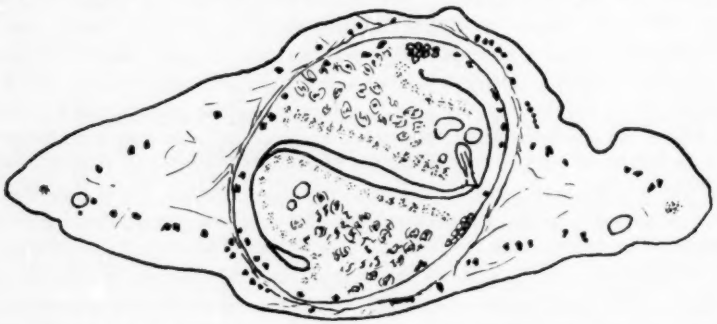


FIG. 1. Cross-section through caudal appendage of completely invaginated plerocercoid of *Pelichnibothrium speciosum* (from a photograph). All longitudinal muscles figured; distribution of testes, ovary, and vitellaria indicated.

Genus *Pelichnibothrium* Monticelli 1889 emended. Scolex with large phyllidia each bearing an auxiliary acetabulum on anterior margin. Genital atria marginal. Vaginal pore anterior to male pore. Vitellaria extending almost to mid-line from each side. Nerve cord and excretory ducts internal to vitellaria. Ovary dumbbell-shaped in surface view and in transverse section. Mehlis' gland posterior and dorsal to ovarian isthmus. Vagina dorsal to uterus. Uterine duct dorsal to vagina. Seminal receptacle present. Entire ventral surface of uterus in contact with cuticle. Outer longitudinal muscle layer weakly developed and contiguous with circular layer. Internal longitudinal muscles internal to vitellaria. Plerocercoids strobilate with a long caudal appendage into which strobila and scolex can be withdrawn; reproductive organs present in all proglottids.

Type species: *Pelichnibothrium speciosum* Monticelli 1889, from *Alepisaurus ferox*.

DISCUSSION

The scolex of *Pelichnibothrium* is that of a phyllobothriid, but the ovary and musculature, the relationship of the vitellaria and uterus to the longitudinal muscles, and the nerve lying internal to the vitellaria, makes it impossible to place the species at present. But there is a remarkable similarity in arrangement of organs to some of the aberrant proteocephalids described by Woodland (1933).

Upon the assumption that the adult stage probably existed in the definitive host

but a short period of time, as in *Ligula* and *Schistocephalus,* and since Smyth (1946) had demonstrated the significance of the temperature factor in inducing maturation in *Schistocephalus,* specimens were placed in sea water, normal saline, and 1% peptone broth at 39°C, 13°C, 7°C, and at room temperature (*cir.* 22°C) in an effort to determine the kind of definitive host. All of the specimens in sea water died within 24 hours, as did all those incubated at 39°C. Those in normal saline and those in peptone broth died in ten days at the lower temperatures, although the solutions were changed daily. Proglottids were not distinctly visible in fresh, living specimens, but after 24 hours in the laboratory, they became separated from one another, and maintained continuity with the chain only by the inner longitudinal muscles. A dish containing several specimens in peptone broth was kept at room temperature on one occasion, and the proglottids separated completely from one another, the testes matured, and the uterus filled with vitelline cells which were discharged by dehiscence. These proglottids were bluntly rounded anteriorly and posteriorly and were 2.7–3.0 mm. long by 2.22–2.25 mm. in the living condition as they moved about in the dish.

It would seem unlikely that the definitive host of the worm could be a bird, since the worms died so rapidly at 39°C, but these experiments actually yielded very little information. If this animal normally parasitizes *Prionace glauca* as indicated by Yamaguti's work, it has a peculiar life-history pattern. *Prionace glauca* was the most frequently autopsied shark from Monterey Bay, California during four years of work on cestodes of elasmobranch fishes and *Pelichnibothrium* was never encountered in any species of shark.

References Cited

Joyeux, C. and Baer, J. G. 1936 Cestodes. *Faune de France,* Féd. Franc. Soc. Sc. Nat. (30) 613 pp.

Monticelli, F. S. 1889 Notes on some Entozoa in the collections of the British Museum. Proc. Zool. Soc. London (21–22) pp. 321–325.

Smyth, J. D. 1946 Studies on tapeworm physiology. I. The cultivation of *Schistocephalus solidus in vitro. J. Exp. Biol., London* **23:** 47–70.

Woodland, W. N. F. 1933 On the anatomy of some fish cestodes described by Diesing from the Amazon. Quart. J. Micr. Sci. **76:** 175–208.

Yamaguti, S. 1934 Studies on the helminth fauna of Japan. Pt. 4. Cestodes of fishes. Jap. Journ. Zool. **6:** 1–112.

Zschokke, F. and Heitz, A. 1914 Entoparasiten aus Salmoniden von Kamtschatka. Rev. Suisse Zool. **22:** 195–255.

RESEARCH NOTE

HOPLOPLEURA OENOMYDIS FERRIS IN PUERTO RICO

Considerable interest has been shown in the occurrence of the tropical rat louse *Hoplopleura oenomydis* Ferris on domestic rats in the United States (Pratt and Karp, 1953, J. Parasit. **39:** 495–504; Ferris and Stojanovich, 1951, Pacific Coast Ent. Soc. Mem. **1:** 1–320; Pritchard, 1947, J. Parasit. **33:** 374–375). In correspondence Dr. Pratt kindly advises me that as far as he knows, this species has not been previously found in Puerto Rico. An ectoparasite survey of more than a thousand rats collected at San Juan, P.R. in 1946–1949 failed to reveal this species although *Polyplax spinulosa* (Burmeister) was quite common (Fox, 1951, J. Parasit. **37:** 85–95). Another rat-ectoparasite survey was made in San Juan from April 15, 1954 to September 1, 1955 in which 748 living domestic rats were collected. From a single *Rattus rattus* captured in the district known as Puerta de Tierra on February 17, 1955 one female and two nymphs of *H. oenomydis* were taken. *P. spinulosa,* however, was much more common, infesting 15.4 percent of the rats with an average number of 0.73 specimens per rat. Although apparently very rare in San Juan, it is possible that *H. oenomydis* occurs more frequently on rats in other parts of Puerto Rico.—Irving Fox, *School of Medicine-School of Tropical Medicine, University of Puerto Rico, San Juan, P.R.*

OCCURRENCE OF THE CESTODE *MATHEVOTAENIA SURINAMENSIS* (COHN, 1902) SPASSKII, 1951 IN A NORTH AMERICAN ARMADILLO[1]

G. D. BUCHANAN[2]
Biology Department, Rice Institute
Houston, Texas

In conjunction with reproductive studies conducted in these laboratories, helminthological examinations were made on about 30 nine-banded armadillos (*Dasypus novemcinctus mexicanus*). Half of these armadillos came from the oak-pine forest 12 miles north of Huntsville, Texas, the others from a ranch 15 miles south of Huntsville. This latter area is primarily cut-over oak-hickory forest, the wooded areas alternating with uncultivated pasture land on which some cattle are ranged. In 2 animals from each locality, cestodes were recovered from the small intestine of sub-adult and, in one case, adult females. The infections ranged from 3 to 8 worms, averaging 5 worms per animal. In one case 6 immature strobilae were recovered which apparently represented two or three separate infections.

The cestodes have proved to be *Mathevotaenia surinamensis*. While the number of testes, dorsal position of the genital ducts, and cirrus-pouch measurements serve to distinguish *M. surinamensis* from other species of the genus thus far described, no adequate description of this species has been published. The recent reviews and keys to the genus (Baer, 1927; Meggitt, 1934; Hughes, 1940; Spasskii, 1951) contain only the measurements given by Cohn in his original description, based on a single strobila. Actually the literature contains only three references to *M. surinamensis*, and two of these concern the same material. It seems desirable, therefore, to provide a redescription of the species based on the material obtained by us. All measurements are in millimeters, unless stated otherwise.

Mathevotaenia surinamensis (Cohn, 1902) Spasskii, 1951

Description: Mature strobila 290 to 400 long. Width increasing from 0.44 to 0.60 just behind scolex to maximum of 2.0 to 2.5 about 2/3 of length from anterior end. Scolex 580 to 800 microns in diameter. Suckers 220 to 280 microns in diameter. Neck short; segmentation beginning 2 to 3 behind scolex. Immature segments broader than long, mature segments roughly square, ripe segments longer than broad. Mature segments 1.03 to 1.60 long and 1.49 to 1.75 broad. Ripe segments 1.95 to 3.20 long and 1.71 to 2.50 broad. Genital primordia first visible approximately 12 posterior to scolex. Genital atria irregularly alternating, about 100 to 140 microns in depth, situated 1/4 to 1/3 length of proglottid from anterior end. Genital ducts dorsal to longitudinal excretory canals, latter extensively developed. Ventral excretory canals 6 to 9 in number, uniformly distributed across proglottid, with numerous anastomoses and transverse commissures, not confined to any particular region of proglottid. Dorsal excretory canals similarly developed but with fewer canals, somewhat laterally disposed. Excretory canals degenerating in ripe segments, diameter of ventral canals becoming irregular and giving appearance of being collapsed. Dorsal canals becoming interrupted and finally disappearing. Broken ends of dorsal canals surrounded by small clumps of cells.

Testes 60 to 80 microns in diameter, 90 to 110 in number, round, posterior and lateral to ovary, extending to, but not beyond ventral lateral excretory canal. Cirrus pouch 300 to 360 microns long, 180 to 250 microns wide, extending to or beyond dorsal lateral excretory canal.

Received for publication, June 20, 1955.
[1] Aided in part by a grant from the National Science Foundation.
[2] The author wishes to express his sincere appreciation to Dr. A. C. Chandler for his advice and assistance in preparing the manuscript.

Sperm duct passing dorsal to female genitalia, widening into external seminal vesicle just distal to coalescence of vasa deferentia.

Ovary bilobed, each lobe consisting of numerous lobules, situated in anterior median portion of segment, poral lobe about half as large as aporal lobe. Maximum width in mature segments 600 to 700 microns. Vitelline gland a compact mass situated medial and slightly posterior to Mehlis' gland. Vagina situated posterior to cirrus-pouch, passing mediad and enlarging slightly directly anterior to poral lobe of ovary then narrowing to a very fine duct, dorsal to ovary, before joining oviduct. Poral segment of vagina highly glandular. Oviduct an S-shaped tube of broad diameter, passing posteriad from isthmus of ovary, then curving sharply anteriad, to its junction with narrow vagina, and then posteriad again to end at small ootype, surrounded by Mehlis' gland, at center of segment. Eggs encapsulated singly in parenchyma, round to oval, 35 to 60 microns in diameter, oncospheres 20 to 40 microns in diameter, oncosphere hooks 17 to 22 microns long. Eggs extending laterally beyond ventral excretory canals and numbering about 4000 per segment.

Hosts: Armadillos: *Priodontes giganteus, Dasypus novemcinctus, Dasypus* sp.
Location: Small Intestine.
Locality: Surinam, Brazil, Texas.

This material represents a new range record for this cestode and except for the listing by Stiles and Hassall (1894) of *"Taenia* sp. from *Dasypus 9-cinctus,"* is the only record of a tapeworm from North American armadillos. The author has examined Stiles and Hassall's material which consists of about a dozen fragments of small unarmed cestodes. The state of preservation is not very good, the material appearing to have been dried at one time. As dehydration caused distortion of the worms, stained specimens were examined in glycerin mounts. Internal structures are not visible, but the following measurements were obtained: scolex, 233 microns; suckers, 100 microns; segmentation beginning 1.14 mm. behind scolex. The above measurements give no useful clue as to the identity of the worms; however, they do indicate that the material does not represent *Mathevotaenia surinamensis.*

Cohn (1902) described *Mathevotaenia* (syn. *Oochoristica*) *surinamensis* from a single worm which Creplin had recovered from a giant armadillo (*Priodontes giganteus*) in Surinam. Cohn noted that his worm was so thick that serial sections had to be made to determine the internal structure. This fact, the short strobila length, and the fact that even his ripe segments were much broader than long, all suggest that his specimen was much contracted. Von Janicki (1904, 1906) recorded the worm from a Brazilian *Dasypus novemcinctus.* His material consisted of fragments of what probably represented two strobilae. Von Janicki (1906) also identified 10 cestodes, which had been in the Vienna Zoological Museum since 1825, as *M. surinamensis* from *Dasypus* sp.

Spasskii (1951) has made considerable revision of the genus *Oochoristica* as it formerly existed. He has removed from the genus all the species parasitic in mammals and one species parasitic in a lizard, leaving *Oochoristica* to contain those forms with one set of genitalia per segment, alternating genital apertures, testes posterior to (or posterior and lateral to) the female genitalia, acraspedote, parasitic in reptiles. The remaining species are distributed among six other genera of the LINSTOWIINAE. *Oochoristica mephitis* Skinker, 1935 and *O. oklahomensis* Peery, 1939, which have unilateral genital apertures, are placed in the genus *Oschmarenia* Spasskii, 1951. *Oochoristica taborensis* Loewen, 1934 and *O. amphisbaenae* Rudolphi, 1819, which have testes anterior and posterior to the female genitalia are placed in the genera *Cycloskrjabinia* Spasskii, 1951 and *Semenoviella* Spasskii, 1951 respectively.

The remaining species removed from *Oochoristica,* are craspedote forms, para-

sitic in mammals and except for four species are placed in the genus *Mathevotaenia.* The basis for this separation is the presence or absence of a seminal receptacle, *Mathevotaenia* being keyed out as "seminal receptacle absent." The other four species *Oochoristica bivittata* Janicki, 1904 (placed by Spasskii in the genus *Linstowia*); and *O. sandgroundi* (Sandground, 1926) Meggitt, 1934; *O. procyonis* Chandler, 1942; and *O. incisa* Railliet, 1899 (all placed by Spasskii in *Atriotaenia*) are distinguished from *Mathevotaenia* by "the proximal part of the vagina developing into a clearly expressed seminal receptacle." While this distinction holds in this instance, no less than 6 of the 15 species in *Mathevotaenia,* including the type species (*M. symmetrica* (Bayliss, 1927) Akhumian, 1946) do possess seminal receptacles. However, in five species (*M. symmetrica, M. lemuris, M. marmosae, M. skrjabini* and *M. surinamensis*) the seminal receptacle is formed by the oviduct, not the vagina. Such a structure is considered by Spasskii not to be a seminal receptacle "in the true sense of the word." In the sixth species, *Mathevotaenia megastoma* (Diesing, 1850) Spasskii, 1951, the vagina is expansible and "serves as a seminal receptacle." Spasskii points out that in this case the expanded part of the vagina is not large. The examples of *Mathevotaenia surinamensis* examined by the present author have the proximal part of the vagina somewhat dilated in the majority of the mature proglottids. Whether or not this portion of the vagina serves as a seminal receptacle is of course problematical but such a function seems not unreasonable. Certainly the structure is similar to that of *M. megastoma.*

Since Spasskii's statement "seminal receptacle absent" cannot be strictly applied without necessitating removal of some of the species in the genus and the alternative statement ". . . vagina developing into a clearly expressed seminal receptacle" is equally inappropriate; it is necessary to revise Spasskii's key or provide new taxonomic groupings for the species involved. The former course seems much better to the writer and he proposes that the definition of the genus *Mathevotaenia* be altered to read "seminal receptacle not formed by proximal end of vagina or, if formed, not well developed."

There are only two other reports of tapeworms having been found in armadillos. Parona (1901) reported *Mathevotaenia* (syn. *Oochoristica*) *tetragonocephala* from *Dasypus* sp. from Sao Paulo, Brazil. Von Janicki (1906) re-examined this material, decided specific identification was not possible, and listed the worm as *Mathevotaenia* (syn. *Oochoristica*) sp. Cameron (1939) reported *Mathevotaenia* (syn. *Oochoristica*) sp. from *Dasypus novemcinctus* in Trinidad.

The above represents the sum of our knowledge of tapeworms in armadillos, and is also the sum of the references to *Mathevotaenia surinamensis* except for the listing by several authors of this worm from *Didelphis marsupialis,* (Baer, 1927; Meggitt, 1934; Hughes, 1940). Baer seems to have been the first to list the opossum as a host for *M. surinamensis,* but none of the references cited by him contains any report of the worm in marsupials. Spasskii (1951) listed *Didelphis aurita* as well as *D. marsupialis* as hosts for *M. surinamensis,* however, a check of these authors' references as well as the literature fails to reveal any report of *M. surinamensis* being found in any animal except the species of armadillo listed above. It seems probable that Baer's listing is an error and that subsequent authors have copied his mistake.

This paucity of data concerning helminths of the armadillo is not confined to cestodes. Chandler (1946) in some armadillos examined by him noted a striking

lack of helminths requiring an arthropod intermediate-host. The review of Talmage and Buchanan (1954) lists only five adult helminths from the DASYPODIDAE requiring such hosts. Four of these, including the cestodes discussed above, are from *Dasypus novemcinctus,* but the material in all cases has come from South America, chiefly Brazil. No explanation of this phenomenon is apparent since by reason of its insectivorous diet and environmental relationships, the armadillo would seem to be pre-eminently suited as a host for parasites utilizing arthropod intermediate hosts. The review mentioned above offers the conjecture that the low and somewhat unstable body temperature of the armadillo might in part explain its lack of parasites. It should be noted, however, that no great amount of work has been devoted to the parasitic fauna of this animal, and that the real deficiency may lie, in part, in the paucity of investigation done on this mammal.

REFERENCES CITED

BAER, J. G. 1927 Monographie des cestodes de la famille des Anoplocephalidae. 241 pp. Suppl. au Bull. Biol. de France et de Belgique, Suppl. 10.

CAMERON, T. W. M. 1939 Studies on the endoparasitic fauna of Trinidad mammals. VI. Parasites of edentates. Canad. J. Research, Sect. D, Zool. Sci. **17**: 249–264.

CHANDLER, A. C. 1946 Helminths of armadillos, *Dasypus novemcinctus,* in eastern Texas. J. Parasit. **32**: 237–241.

COHN, L. 1902 Helminthologische Mittheilungen. Arch. Naturg., Berlin **69**: 47–68.

HUGHES, R. C. 1940 The Genus Oochoristica Lühe 1898. Am. Midland Nat. **23**: 368–381.

VON JANICKI, C. 1904 Zur Kenntnis einiger Säugetiercestoden. Vorläufige Mitteilung. Zool. Anz. **27**: 770–782.

———— 1906 Studien an Säugetiercestoden. Ztschr. Wissensch. Zool. **81**: 505–597.

MEGGITT, F. J. 1934 On some tapeworms from the bullsnake (*Pityopis sayi*), with remarks on the species of the genus *Oochoristica* (Cestoda). J. Parasit. **30**: 181–189.

PARONA, C. 1901 Di alcuni cestodi brasiliani, racolti dal Dott. Adolfo Lutz. Boll. Mus. Zool., Genova (102), 12 pp.

SPASSKI, A. A. 1951 Anoplotsefaliaty-Lentochnye Gel'minty domashnikh i dikikh zhivotnykh. Osnovy tsestodologii 1, pp. 735, Akademia Nauk, SSSR, Moskva. (Russian text). (Anoplocephalata-Cestodes of domestic and wild animals.)

STILES, C. W. AND HASSALL, A. 1894 A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biological Department of the University of Pennsylvania (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. Vet. Mag. **1**: (4) 245–253; (5) 331–354.

TALMAGE, R. V. AND BUCHANAN, G. D. 1954 The Armadillo. Rice Inst. Pamphlet **41** (2), 135 pp.

PLATE I

Mathevotaenia surinamensis (Cohn, 1902) Spasskii, 1951

FIG. 1. Mature segment of *Mathevotaenia surinamensis.* (×85)

FIG. 2. Dorsal longitudinal excretory canals in mature segment of *Mathevotaenia surinamensis.* (×85)

FIG. 3. Ventral longitudinal excretory canals in mature segment of *Mathevotaenia surinamensis.* (×85) Figures 1, 2, and 3 taken from same segment.

FIG. 4. Ripe segment of *Mathevotaenia surinamensis.*

FIG. 5. Scolex of *Mathevotaenia surinamensis.* (×60)

FIG. 6. Egg and oncosphere of *Mathevotaenia surinamensis.*

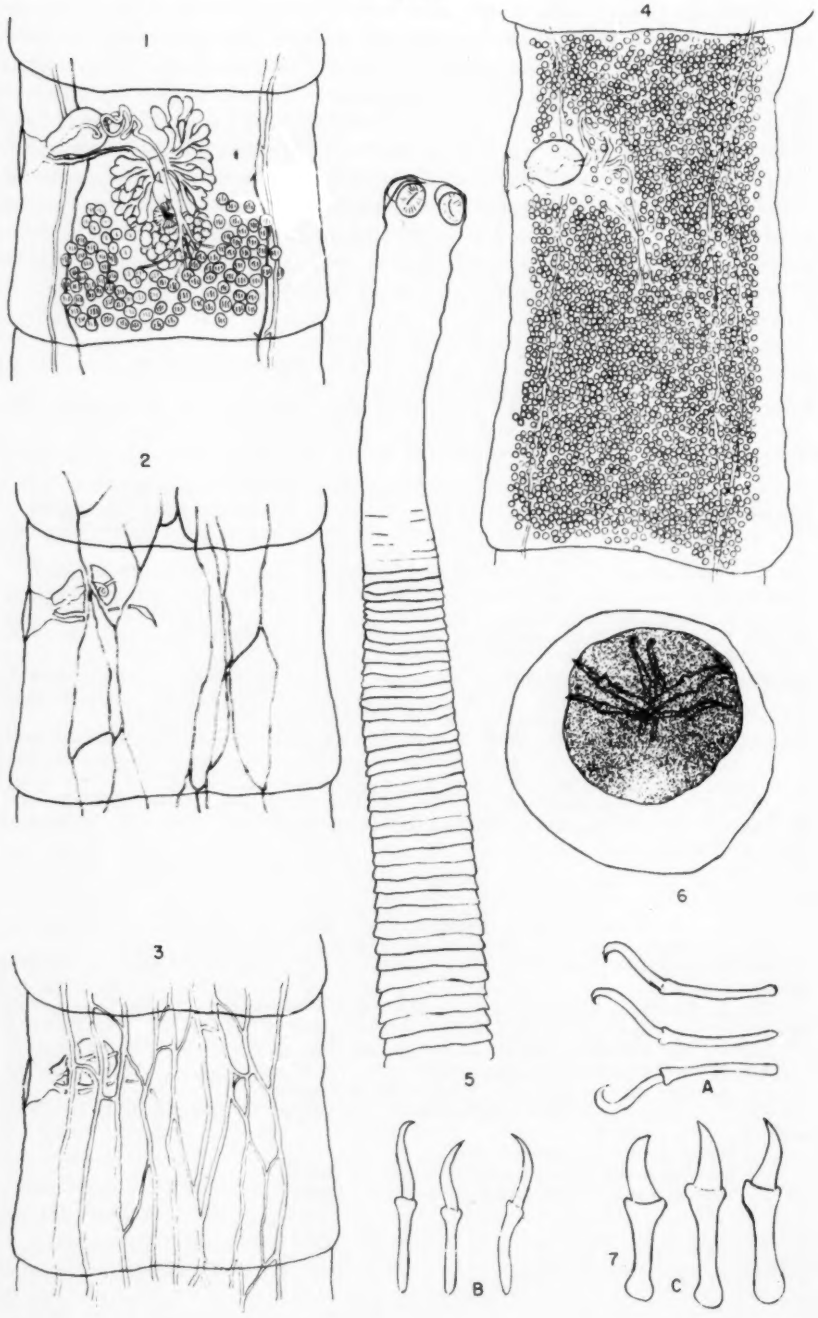
FIG. 7. Oncosphere hooks of *Mathevotaenia surinamensis* showing variations in size and shape.

a). Medial pair of oncosphere hooks.

b.) External hooks of lateral pair of oncosphere hooks.

c.) Internal hooks of lateral pair of oncosphere hooks.

PLATE I



A NEW SPECIES OF *CENTRORHYNCHUS* (ACANTHOCEPHALA) FROM THE KITE, *MILVUS MIGRANS*, IN EGYPT

HELEN L. WARD
The University of Tennessee, Knoxville

In 1952 and 1953 a number of Acanthocephala belonging to the genus *Centrorhynchus* were collected from the kite, *Milvus migrans*, by the department of helminthology, United States Naval Medical Research Unit No. 3, Cairo, Egypt, and forwarded to the writer by Dr. Robert E. Kuntz. These parasites differ from previously described species of this genus in the number and arrangement of proboscis hooks and in other respects, and are therefore described here as a new species.

Centrorhynchus milvus n. sp.
Figs. 1–5
(All measurements in millimeters)

Description: With the characters of the genus *Centrorhynchus*. Body cylindrical with anterior fourth distinctly swollen. Females 24 to 32 by 1, anterior swollen region 2 in diameter. Posterior tip of female (Fig. 2) with small rounded projection. Males (Fig. 1) 16 to 20 by 1, anterior swollen region 1.5 in diameter. Anterior part of proboscis vase-shaped, posterior part truncate, broader than anterior part. Length of proboscis 0.8 to 1.1; diameter 0.4 to 0.5 at base; slightly larger in females than in males. Proboscis hooks (Fig. 3) in 28 to 32 longitudinal rows with 18 to 21 in each row. Anterior 8 or 9 hooks of each row large, with heavy roots. Dorso-ventral differentiation (Fig. 4, a and b) in these anterior hooks pronounced. Length of ventral hooks 0.04 to 0.05 with roots about equaling length of hooks. Dorsal hooks smaller, 0.02 to 0.03 long with roots much longer than hooks, about 0.04 to 0.05. Posterior to latter hooks on swollen basal region of anterior part of proboscis 5 or 6 hooks (Fig. 4c) measuring 0.025 to 0.030 long with Y-shaped roots slightly shorter than hooks and extending anteriorly from hooks. Five or six spines (Fig. 4d) on posterior part of proboscis measuring 0.015 to 0.020, lacking roots. Proboscis sheath 1.3 to 1.6 by 0.30 to 0.40. Lemnisci somewhat longer than sheath, about 1.8 to 2.8 by 0.20 to 0.30. Mature embryos (Fig. 5) in gravid females elliptical, averaging 0.05 by 0.02. Acanthor approximately 0.03 by 0.012 with large nuclear mass in center and hooks at anterior extremity. Outer and inner shells well developed. Inner membrane surrounding acanthor slightly thickened at poles. Testes in anterior swollen region of males, about 0.8 to 1.1 by 0.3 to 0.5. Cement glands very long, 8 to 10 by 0.2 to 0.3

Type host: Milvus migrans (kite).

Locality: Lake Qarun, Faiyum, Egypt.

Type material: Holotype male, No. 37495, and allotype female, U. S. N. M. Helm. Coll. Paratypes representing both sexes in Parasitology Collection, Department of Zoology and Entomology, University of Tennessee, and Department of Parasitology, U. S. Naval Medical Research Center, Bethesda.

Numerous species of the genus *Centrorhynchus* have been described from *Milvus* sp. and other falconiform birds from various parts of the world (Meyer, 1932). *C. buteonis* (Schrank, 1788) has been reported from *Milvus ictinus, Buteo swainsoni,* and other hosts in Europe; this species differs from *C. milvus* in having a larger body and fewer hooks in each longitudinal row. *C. globocaudatus* (Zeder, 1800) from *Milvus milvus* and other hosts in Europe and Africa differs from the new species in the shape of the body and of the proboscis. *C. magnus* Fukui, 1929 from *Milvus lineatus* and *Falco tinnunculus* in Japan differs from the new species in having a larger body and more numerous proboscis hooks. *C. albidus* Meyer, 1932 has been reported from Falconidae in South America; this species is characterized by a smaller body than the new species. *C. aluconis* (Mueller, 1780) has

Received for publication, August 8, 1955.

been reported from *Strix aluco, Falco tinnunculus* and other hosts in Europe; the body of this species is not swollen anteriorly and the number of hooks in each longitudinal row is smaller than that of *C. milvus*. *C. tumidulus* (Rudolphi, 1819) has been reported from *Crotophaga ani, Leucopternis albicollis* and other hosts in Brazil; the body of this species does not possess the anterior swelling characteristic of *C. milvus*. *C. polymorphus* Travassos, 1926 from *Micrastur ruficollis* in Brazil, differs from the new species in having a club shaped body and a conical proboscis. *C. asturinus* (Johnston, 1916, 1918; Johnstone and Deland, 1929) from *Accipiter novaehollandiae* and other hosts in Australia differs from the new species in possessing more numerous proboscis hooks.

Das (1950a) reported *C. falconis* from *Circus macrourus* in India; this species differs from the new species in the shape of the proboscis and in having a larger number of hooks (12–14) in each longitudinal row on the anterior region of the proboscis. Das (1950b) reported two juvenile forms, *C. amphibius* from *Ptyas mucosus* and *Rana tigrina* and *C. microcervicanthus* from *Naia tripudians*, both of which possess about the same number of hooks as the new species. However, the size of the hooks in both of these species is distinctly smaller than that of the new species.

Literature Cited

Das, E. N. 1950a On some new species of the genus *Centrorhynchus* Lühe, 1911. Rec. Ind. Museum **47**: 291–301.

———1950b On some juvenile forms of Acanthocephala of the genus *Centrorhynchus* from India. Ind. J. Helminthol. **2**: 49–56.

Meyer, A. 1932 Acanthocephala. Bronn's Klass. u. Ordnung. Tier-reichs, 4, Bd., 2 Abt., 2 Buch, 1 Lief., pp. 1–332.

Plate I

Explanation of Figures

All figures are of *Centrorhynchus milvus* n. sp.

All drawings made with the aid of microprojection from stained whole mounts.

Fig. 1. Adult male.

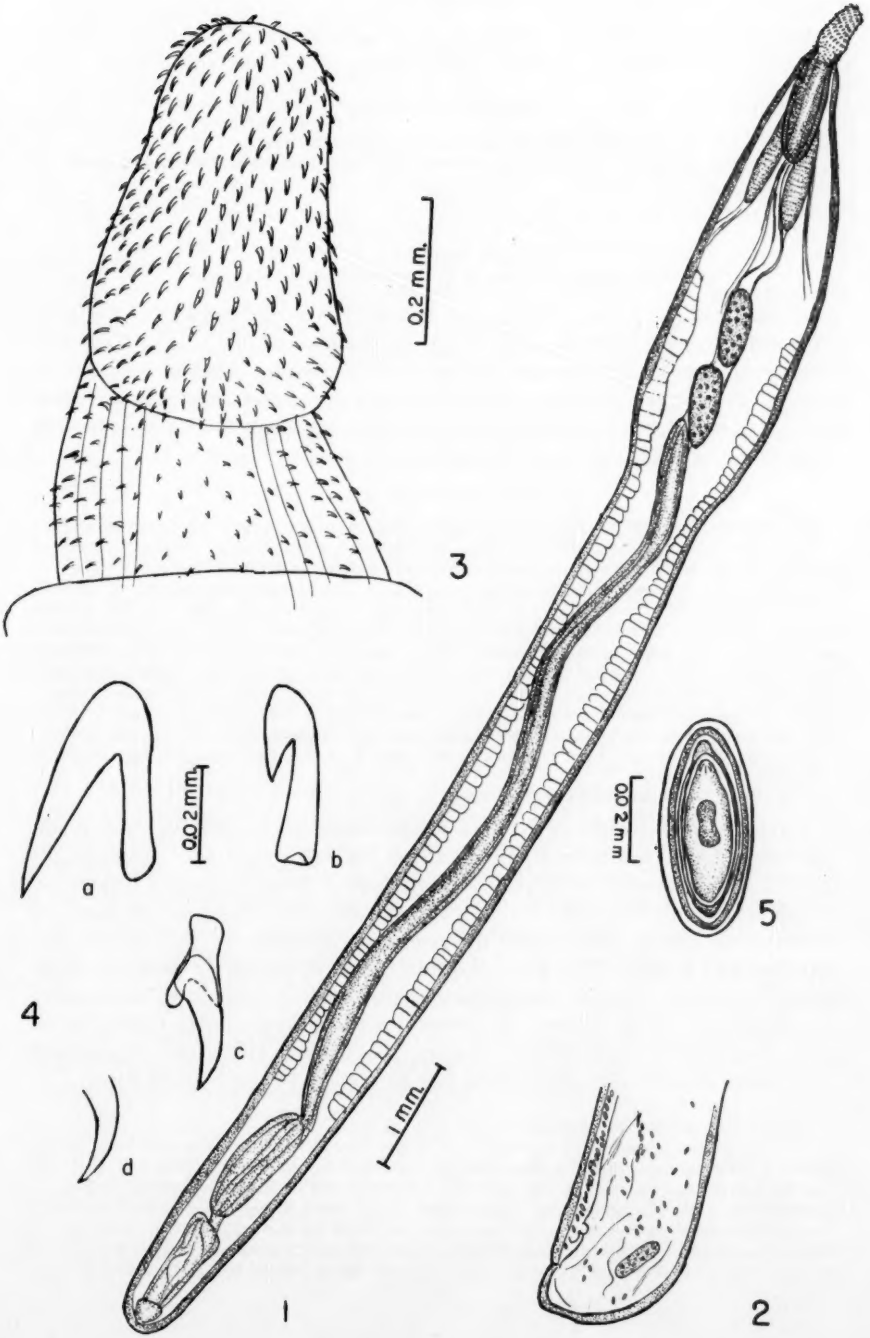
Fig. 2. Posterior extremity of female.

Fig. 3. Proboscis.

Fig. 4. Hooks from proboscis: a, ventral hook from anterior part of proboscis; b, dorsal hook from anterior part of proboscis; c, hook from basal region of anterior part of proboscis; d, hook from posterior part of proboscis.

Fig. 5. Hard-shelled embryo.

PLATE I



LOPHORTOFILARIA CALIFORNIENSIS N. G., N. SP. (FILARIOIDEA, DIPETALONEMATIDAE) FROM CALIFORNIA QUAIL, *LOPHORTYX CALIFORNICUS*, WITH NOTES ON ITS MICROFILARIA

EVERETT E. WEHR

Animal Disease and Parasite Research Branch
Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

AND

CARLTON M. HERMAN

Fish and Wildlife Service
United States Department of Interior, Laurel, Maryland

A number of filarioid nematodes were recovered from the ventricles of the heart of the California quail during a survey of the parasites of this host in California several years ago.* Unfortunately, before these nematodes could be identified, a fire swept through the building in which they were stored and completely destroyed many of them. A recent study of the few remaining specimens showed them to represent a new genus and a new species.

Lophortofilaria new genus

Description: Dipetalonematidae: Body more or less uniform in width, not distinctly swollen in region of excretory sinus, and only slightly attenuated at extremities. Cuticle devoid of vesiculate bosses and transverse thickenings, but with distinct longitudinal and cross striations. Oral opening not surrounded by cuticular projections. Number and arrangement of cephalic papillae not determinable in specimens available for study. Buccal capsule much reduced. Esophagus not divided into anterior muscular and posterior glandular parts. *Male* with spicules short and subequal. Two pairs of caudal papillae present, one pair anterior and the other posterior to anal opening. Tail short and bluntly rounded. *Female* with posterior end slightly narrowed. Anus apparently atrophied; large vacuolated area present in vicinity where anal opening usually occurs, but without external opening. What appears to be a narrow canal, connects above-mentioned vacuolated area with another smaller vacuolated area near tip of tail, but likewise with no external opening. First stage larva surrounded by thin shell only, without denticles or cuticular spines.

Type species—Lophortofilaria californiensis n. sp.

The genus *Lophortofilaria* is placed in the family DIPETALONEMATIDAE Wehr, 1935 because of the character of the egg and of the first stage larva. According to Chabaud and Choquet's (1953) recent classification of the superfamily FILARIOIDEA, this genus falls into the subfamily SPLENDIDOFILARIINAE. Based on the length of the tail, these authors have divided the genera of the subfamily SPLENDIDOFILARIINAE into two groups. The genus *Lophortofilaria* is tentatively assigned to the short tailed group, since the anal opening of the female is believed to be atrophied or subterminal. If this is true, the present genus is most closely related to the genus *Aproctiana* Skrjabin, 1934, (Skrjabin, 1934) from which it may be separated by the fewer number of caudal papillae and by spicules of nearly equal length.

Received for publication, August 17, 1955.

* Credit is due a number of the personnel of the then Division of Fish and Game of the State of California, particularly to Ben Glading and David Selleck, who in 1941 and 1942 supplied the infected quail as part of the activities of Federal Aid in Wildlife Restoration Project, California 6R (The Management of Valley Quail in the South Coast Counties of California). The experimental work reported under occurrence was done by the junior author when he was Technical Advisor of the Los Angeles Wildlife Disease Research Station of the U. S. Fish and Wildlife Service (in charge of Works Projects Administration Official Project No. 65-2-07-344).

Lophortofilaria californiensis new species
(Measurements in millimeters.)

Specific diagnosis—Same as for genus.

Male: 6 to 8 long and 0.158 wide near middle of body. Esophagus 0.150 long, narrow. Long spicule 0.68–0.78 in length, slightly twisted in its posterior half and bluntly pointed distally. Short spicule 0.56–0.71 long, with anterior half slightly broader than posterior, distal end distinctly enlarged. Tail 0.88 long. Two pairs of caudal papillae, one pair distinctly anterior to, and other directly behind, anal opening.

Female: 15 to 18 long and 0.244 wide near middle of body. Exact length of esophagus not determinable because union with intestine obscured by uterine coils; however, estimated length about 0.367. Vulva 0.290–0.308 from anterior end of body. Vagina 0.610–0.638 long, dividing into two posteriorly directed uteri. No anal opening detectable; if present, possibly subterminal. Tail about 243 microns long.

First stage larva 0.126 long and 0.03 wide, slightly stouter at anterior end and devoid of spines or denticles.

Type host: California quail (*Lophortyx californicus*). Collected from both subspecies, *Lophortyx c. californicus* and *L. c. vallicola.*

Location—Ventricles of heart, particularly at auriculo-ventricular valve.

Type locality—Bitterwater, San Benito County, California.

Type specimens—(male and female) U. S. Nat. Mus. Helminthological Collection, No. 48463.

Because of the poor condition of the material, such questions as the number and position of the cephalic papillae, exact position of vulva in relation to union of esophagus and intestine, the presence or absence of anal opening, must await study of other specimens.

Observations on occurrence of microfilariae of *Lophortofilaria californiensis*

Microfilariae were rarely observed in the peripheral blood smears of infected quail. In such birds usually only a few embryos were present and no evidence of periodicity was observed. Microfilariae could usually be demonstrated in great numbers in blood from the heart of infected birds. During September 1941 an experiment in which six quail, known to be infected by examination of heart puncture smears, were bled from the heart every four hours for a period of three days. Two of these birds died on the second day as a result of the technique employed and/or loss of blood. One large drop of blood was removed by heart puncture from each bird at every 4-hour interval. This provided sufficient material for preparation of a fresh smear in saline and for a dried blood film which was stained with Giemsa's stain. Counts were made of the total number of microfilariae on entire stained smears. By rough approximation these smears were considered samples of similar size. Although, during the three day period of the study the numbers of microfilariae varied, they were no more numerous during evening or night than during the daylight hours and there was no evidence of any periodicity.

SUMMARY

A new genus and a new species of a filariod nematode, *Lophortofilaria californiensis,* from the California quail, *Lophortyx californicus,* has been described, with observations on periodicity of its microfilariae.

REFERENCES CITED

CHABAUD, A. G. AND CHOQUET, M. T. 1953 Nouvel essai de classification des filaires (superfamily des Filarioidea). Ann. Parasitol. **28:** 172–192.

SKRJABIN, K. I. 1934 *Aprocta semenovi* n. sp., nouveau nématode de l'oeil de l'engoulevent. Ann. Parasitol., v **12** (5): 362–366.

WEHR, E. E. 1935 A revised classification of the nematode superfamily Filarioidea. Proc. Helminth. Soc. Washington **2:** 84–88.

PLATE I

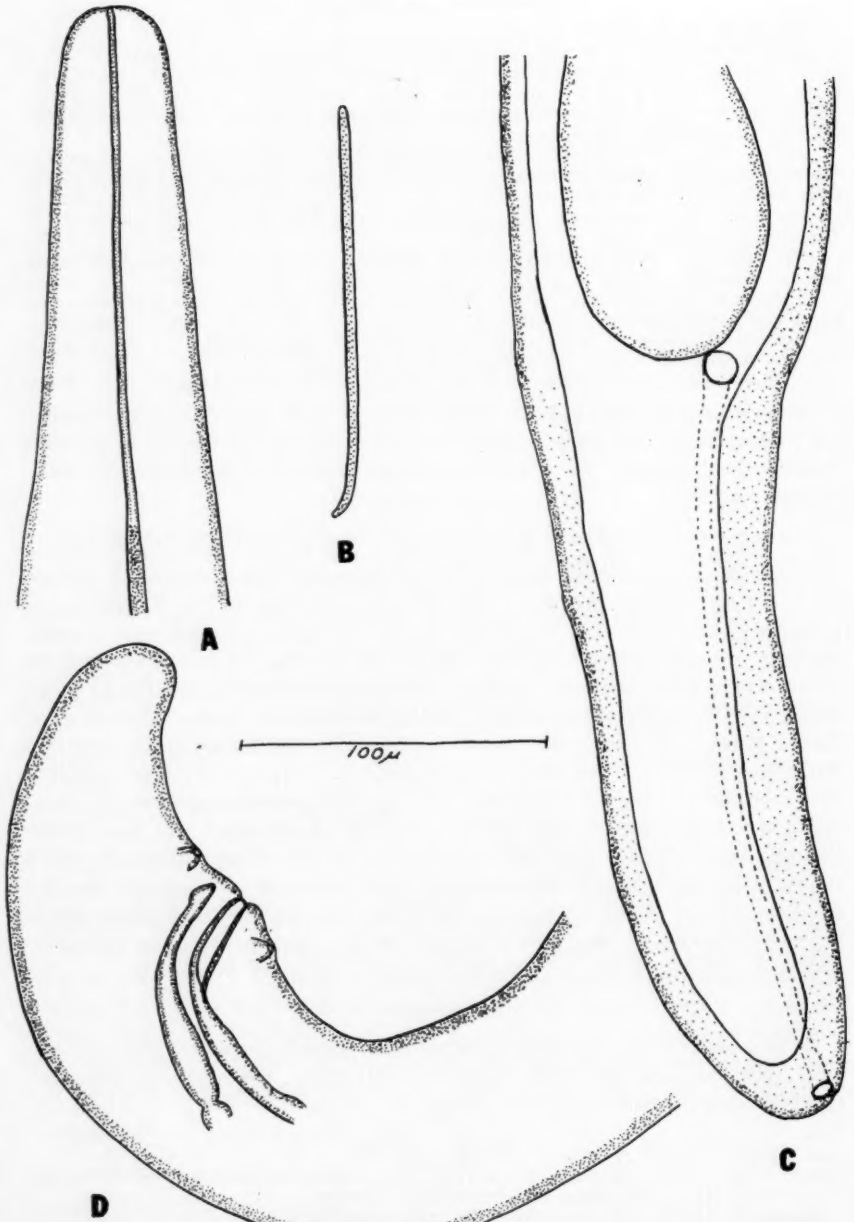


PLATE 1. *Lophortofilaria californiensis* n. g., n. sp. A Male. Anterior end showing esophagus. B Microfilaria. C Female. Posterior end. D Male. Posterior end showing spicules and papillae.

TROGLOSTRONGYLUS WILSONI (STOUGH, 1953) N. COMB. (NEMATODA: METASTRONGYLIDAE) FROM THE LUNGS OF THE BOBCAT, *LYNX RUFUS RUFUS*

LUZ SARMIENTO AND B. D. STOUGH*

Animal Pathology Section, Virginia Agricultural Experimental Station
Blacksburg, Virginia

During the winter months of 1952, the Virginia Cooperative Wildlife Unit collected for study a number of bobcats, *Lynx rufus rufus* (Shreber), from Virginia and North Carolina. These animals were preserved in formalin. The lungs of 25 percent of 64 bobcats examined were found to be heavily infected with the nematode lungworm here described.

The worms were located in the bronchial tubes, bronchioles and lung tissues. None were found in the pulmonary blood vessel. The specimens were transferred to 70% alcohol as they were collected. Some were dehydrated in alcohols, using absolute alcohol at the end of the series, and cleared in beechwood creosote; these were stained by adding a small amount of either fast green or carmine to the clearing agent. The measurements and drawings were made, with the aid of a calibrated ocular micrometer and a camera lucida from 24 specimens cleared in Lactophenol.

The worms here described belong in the family METASTRONGYLIDAE and in the genus *Troglostrongylus*.

Troglostrongylus wilsoni (Stough, 1953) n. comb.**
(Measurements in millimeters)

Description: Head rather distinctly set off from cervical region where cuticle tends to be slightly inflated and thrown into folds. Stoma very small and weakly developed. Oral opening more or less circular, surrounded by 6 lips, 2 dorso-submedians, 2 laterals, and 2 ventro-submedians. Submedian lips more distinct, larger and taller than lateral ones, their apical margins covered with relatively thick cuticle. Five pairs of sensory organs of external circle, one pair at postero-lateral margin of each submediam lip, not on lips but located very near their bases, and one very small papilla ventral to amphids on each lateral lip. Three pairs of sensory organs, one papilla on each lip, in internal circle (Fig. 1). Esophagus relatively short and club-shaped. Nerve ring and excretory pore near middle of esophagus. Cervical papillae slightly anterior to excretory pore (Fig. 2).

Female: 15.5–23.4 long, and 0.34–0.42 wide; esophagus 0.31–0.38 long, and 0.05–0.07 wide at bulb. Vulva situated near middle of body; reproductive system amphidelphic; ovijectoral musculature poorly developed (Fig. 3). Tail more or less conical, length from 0.21–0.26 (Fig. 4). Apparently ovoviviparous; larvae free of shell present in preserved specimens; no living worms observed.

Male: 8.2–12.1 long and 0.31–0.39 wide. Esophagus 0.32–0.38 wide. Bursa symmetrical, relatively small, diameter from 0.21–0.26. Ventral rays small, directed more or less anteriorly; fused except in the distal third to half of their length, from 0.045–0.060 long. Antero-lateral and medio-lateral rays fused first third to half of their length; medio-lateral longer than

Received for publication, September 20, 1955.

* The authors wish to express sincere thanks to Doctor W. L. Threlkeld of Virginia Polytechnic Institute, Blacksburg, Virginia for his assistance and supervision during the course of this work and to Mr. D. G. Progulske for procuring the bobcats that made this study possible. Acknowledgment is made to Mr. J. T. Lucker of Bureau of Animal Industry, for his technical advice.

** A different binomial has previously been applied to this parasite in a brief descriptive note by the junior author (Stough, B. D. 1953). The parasite's trivial name honors Dr. I. D. Wilson, Head of the Biology Department of Virginia Polytechnic Institute.

antero-lateral and nearly reaching bursal margin, length 0.046–0.069 and 0.058–0.080, respectively. Postero-lateral and externo-dorsal rays arising from common base and fused about first fourth of their length, their tips relatively widely separated, length 0.046–0.092 and 0.046–0.067, respectively. Dorsal ray reaching bursal margin; tapering distally, except near slightly expanded tip or bearing 6 small digitate papillae; length about 0.060–0.078 (Figs. 8 & 9). Spicules long, slender and equal; shafts with transversely striated flange except near distal tips; about where cross striation ends, each spicular shaft split into two processes, one relatively long, rather lightly sclerotized (light brownish), curved, sharply pointed, resembling a spur, second stubbier, heavily sclerotized (brownish) ending in bluntly rounded knob. Part of spur invested in thin web or membranous expansion of almost colorless cuticle arising from stubbier terminal process and appearing to be drawn out posteriorly into two sharply tipped wings; latter reaching almost to tip of spur. Length of spicules from 1.70–1.85 (Figs. 5 & 7). Gubernaculum single, though possibly formed by fusion of two lateral elements; more or less boat-shaped in lateral aspect and hastate in ventral aspect. Length about 0.183–0.200 (Fig. 6).

Host: *Lynx rufus rufus.*

Location: Lungs.

Geographic distribution: North Carolina and Virginia.

Type specimens: U. S. Nat. Mus. Helminth. Coll., No. 37434.

Discussion: The more or less equatorial position of the vulva and the amphidelphic reproductive system in the females place this metastrongylid lungworm in the subfamily SKRJABINGYLINAE, Skrjabin, 1933, as constituted by Dougherty (1949) and by Chitwood and Chitwood (1950, p. 18). In all other metastrongylid subfamilies, the vulva is near the anus and the female reproductive system is prodelphic. Dougherty transferred the SKRJABINGYLINAE from the METASTRONGYLIDAE to the TRICHOSTRONGYLIDAE, but he (1951) later took cognizance of Gerichter's (1949) arguments against this placement and acknowledged that this subfamily and all genera he (1949) had included in it, may properly be included in the METASTRONGYLIDAE.

Its bursal ray pattern, the nature of its spicules, and certain other of its characters, exclude the lungworm here described from all presently recognized skrjabingylin genera, except *Troglostrongylus.* In addition to the genotype, *T. troglostrongylus* Vevers, 1923, two species, *T. delicatus* Travassos, 1946 and *T. brevior* Gerichter, 1949, previously have been described in this genus. In these two species, the antero-lateral rays arise independently and the medio-lateral and postero-lateral rays are partly fused, whereas in *T. troglostrongylus,* the antero-lateral and medio-lateral rays are largely fused and the postero-lateral rays are single.

Thus, it is evident that *T. wilsoni* needs to be further differentiated from *T. troglostrongylus,* only. According to Vevers (1923), in the male of his genotype from the frontal sinuses of *Felis bengalensis* the bursal diameter is 0.5 mm. and the postero-lateral and externo-dorsal rays arise singly; the spicules are 2.25 mm. long, presumably in a specimen 12.0 mm. long, and the "dagger shaped" gubernaculum is 0.270 mm. long. Each spicule ends in a "palmate expansion", invested with a cuticular web with minute spines.

In the species here described, the postero-lateral and externo-dorsal rays arise from a common base and are proximally fused for a short distance; in effect all rays except the dorsal, are grouped in pairs. These characters are regarded as sufficient reasons for considering this lungworm specifically distinct from *T. troglostrongylus.* However, although certain of the measurements given by Vevers (1923) do not appear to be consistent with those derivable from his illustrations (*l. c.;* Text-fig. 5), it further must be concluded that the spicules and gubernacu-

lum are proportionately longer than in *T. wilsoni*. Both the structure of the spicules and the shape of the gubernaculum in the present species appear to be further features of difference, so far as can be judged from Vevers' description. Although he describes only two lips in the genotype, whereas six lips are present in the present species, the accuracy of his description of the head of the genotype appears questionable. The presence of a pair of cervical papillae in *T. wilsoni* serve to further differentiate these two species.

Although Dougherty (1949; 1951) has formally proposed the designation *Bronchostrongylus delicatus* and *B. brevior* for the species originally described in *Troglostrongylus* by Travassos and Gerichter, respectively, he (1951) conceded that it might be feasible to synonymize *Bronchostrongylus* with *Troglostrongylus*. Although minor exception to his transfer of these two species to *Bronchostrongylus* may be taken on the ground that the male of each has a well developed gubernaculum, whereas none is described for the male of the genotype, *B. subcrenatus*, recognition of both genera appears convenient for the present. In view of the ray pattern in *T. wilsoni*, Dougherty's (1949) key for separation of the genera may be altered so as to separate *Troglostrongylus* from *Bronchostrongylus* exclusively on the basis of partial fusion of the antero- and medio-lateral rays in the former versus partial fusion of the medio- and postero-lateral rays in the latter.

References Cited

Chitwood, B. G. and Chitwood, M. B. 1950 An introduction to nematology. Baltimore, 2nd Ed.

Dougherty, E. C. 1946 The genus *Aelurostrongylus*, Cameron 1927 (Nematoda: Metastrongylidae) and its relatives; with descriptions of *Parafilaroides*, gen. nov. and *Angiostrongylus gubernaculatus* sp. nov. Proc. Helminth. Soc. Washington **13:** 16–26.

———1949 A list of trichostrongylid lungworms (Phylum Nematoda) and a key to six genera. Parasitology **39:** 218–221.

———1951 A further revision in the classification of the Metastrongylidae Leiper, [1909] (Phylum Nematoda). Parasitology **41:** 91–96.

Gerichter, C. B. 1949 Studies on the nematodes parasitic in the lungs of Felidae in Palestine. Parasitology **39:** 251–262.

Stough, B. D. 1953 A new lungworm parasite from the bobcat, *Lynx rufus rufus*. Abstract of paper presented 14. Ann. Meet. Southeastern Biol. J. Tenn. Acad. Sci. **28:** 189.

Travassos, L. P. 1946 Sôbre um Metastrongylidae das cavidades nasais de *Didelphis marsupialis aurita*. Wied. Rev. Brasil. Biol. **6:** 499–502.

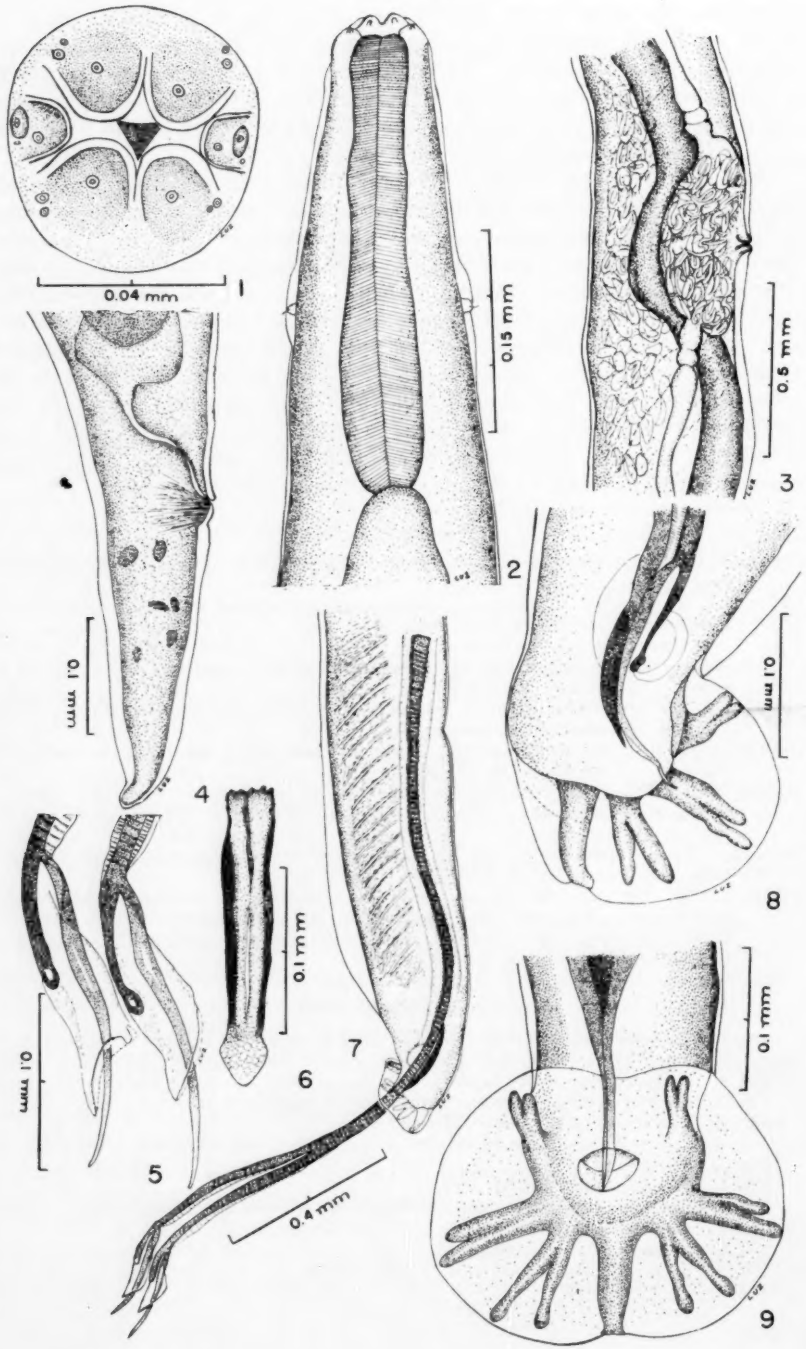
Vevers, G. M. 1923 On the parasitic nematoda collected from mammalian hosts which died in the Gardens of the Zoological Society of London during the years 1919–1921. With a description of three new genera and three new species. Proc. Zool. Soc. London (1922), pt. 4, pp. 901–919.

Explanation of Plate.

All figures were drawn with the aid of a camera lucida and concern *Troglostrongylus wilsoni* (Stough, 1953) n. comb.

Fig. 1. *En face* view of the head.
Fig. 2. Anterior portion of male, showing the esophagus and papillae.
Fig. 3. Vulvar region of female. Lateral view.
Fig. 4. Tail of female. Lateral view.
Fig. 5. Spicule tips.
Fig. 6. Gubernaculum. Ventral view.
Fig. 7. Posterior portion of male, showing bursa, spicules and gubernaculum. Lateral view.
Fig. 8. Bursa of male. Lateral view.
Fig. 9. Bursa, showing all the rays. Ventral view.

PLATE I



NIDIFICATION OF A MITE (*PSORERGATES SIMPLEX* TYRRELL, 1883: MYOBIIDAE) IN THE SKIN OF MICE[1]

ROBERT J. FLYNN AND BERNARD N. JAROSLOW

Division of Biological and Medical Research
Argonne National Laboratory
Lemont, Illinois

When skin flaps were reflected during surgical procedures in mice, small, whitish areas on the inner aspect of the skin were observed (Dr. F. Wassermann, personal communication). Microscopic section showed these areas as pouches in the skin. Dissection of a pouch from unfixed skin revealed the presence of mites that were subsequently identified by Dr. E. Baker of the U. S. Department of Agriculture, as *Psorergates simplex* Tyrrell, 1883. A review of the genus can be found in the paper by Carter (1941).

The pouches were first described by Piana (1886) as nodules on the inner aspect of the skin, the size of a grain of millet, with a small opening to the outer surface. Neumann (1893) confirmed the observations of Piana and noted that the nests were distributed in the skin on all parts of the body but particularly on the face and forehead. Sambon (1928) observed these pouches in association with skin tumors and suggested a causal relationship. In the present investigation we studied different stages in the process of nidification.

MATERIALS AND METHODS

The mice used in these studies were of the CF#1 strain, 4 months of age or older. Groups of 10 to 15 mice were housed in plastic cages bedded with shavings. The cages were changed one to three times a week.

Live mites were obtained by dissecting macroscopic pouches from unfixed skin (Fig. 1). The tissues were fixed in modified Tellyesniczky's fixative (100 parts 70% ethanol, 10 parts formol, and 5 parts acetic acid), sectioned at 6 micra, and stained with hematoxylin and eosin.

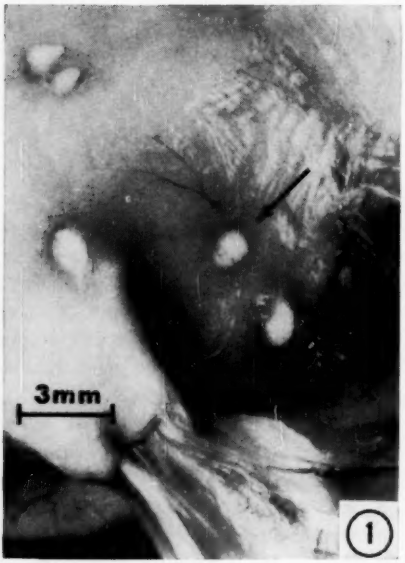
OBSERVATIONS

Pouches were found in the skin on all parts of the body; over the legs, back, thorax, abdomen, shoulders, and particularly the head. The largest pouches, up to 2 mm. in diameter, were found on the back of the neck where the skin is loose, and where growth of the pouch is not restricted by pressure. Conversely, on the legs and face where the skin is tight, mostly small pouches were observed. When a nest was incised and the contents expressed, mites (*Psorergates simplex*) in all stages of development were observed (Fig. 2).

In section, the nests were seen as an invagination of the skin with normal epidermis continuous with the outside. The nest develops from a punctate invagination of the epidermis containing one or few mites (Fig. 3), to a large flask-shaped pouch full of mites and debris (perhaps sloughed, cornified epidermis) as depicted in Figures 4 and 5. In the large nest shown in Figure 5, comparable in size to those in Figure 1, the epidermis was continuous and normal in appearance along the

Received for publication, August 26, 1955.

[1] Work performed under the auspices of the U. S. Atomic Energy Commission.



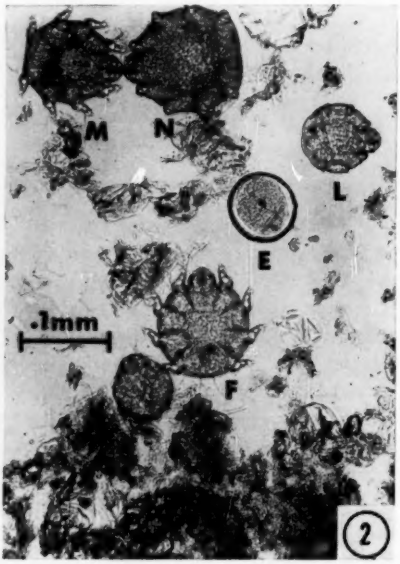
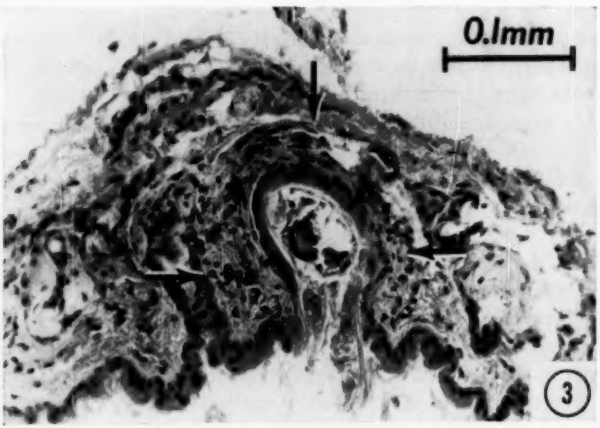


FIG. 1. Inner aspect of mouse skin showing macroscopic pouches (arrow).

FIG. 2. A pouch dissected to show contents; E Egg, F Adult female, L Larva, M Adult male, N Nymph.



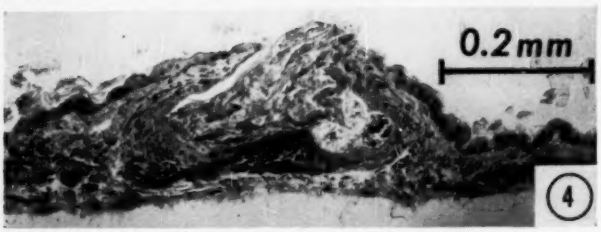


FIG. 3. Early pouch (arrows). Note the normal appearance of the epidermis around the lumen and its continuation with the external epidermis.

FIG. 4. Pouch filled with mites and debris. Considerably larger than early pouch. Epidermal lining still intact and normal.

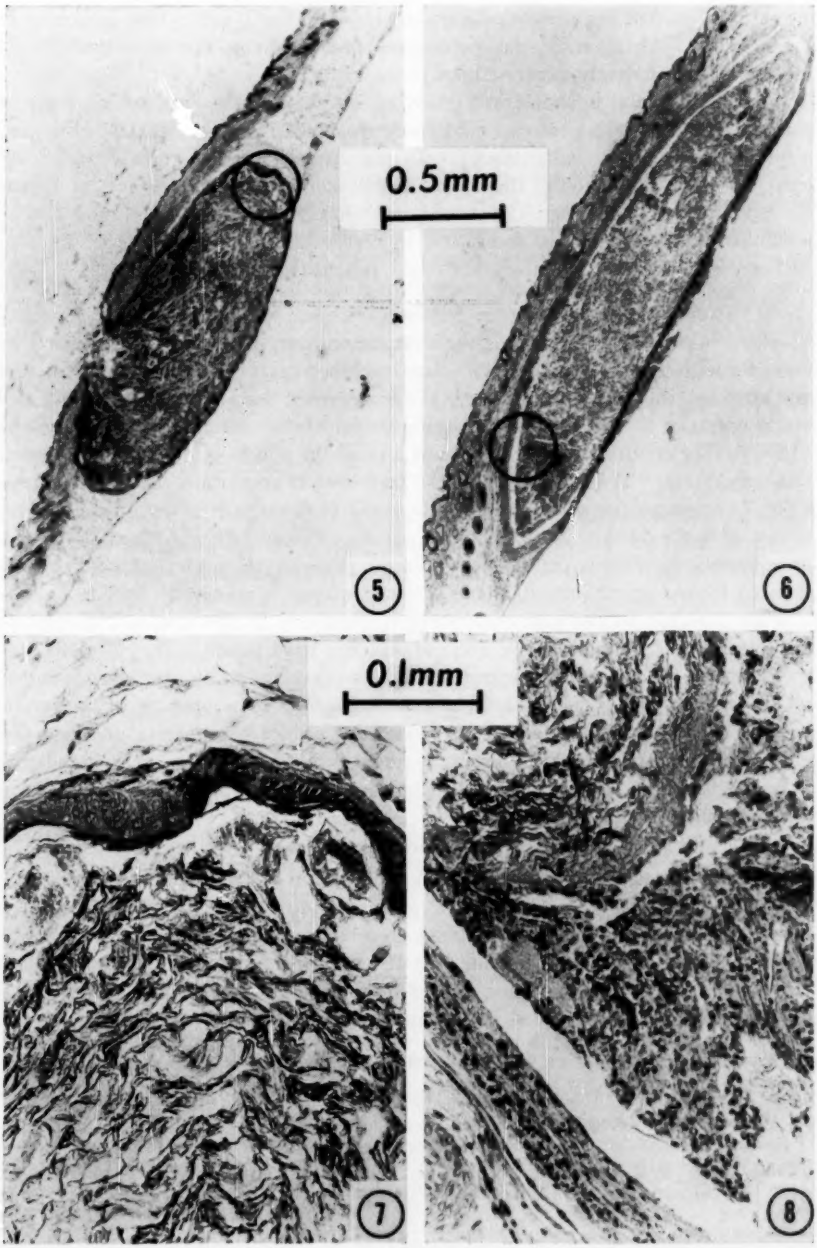


FIG. 5. Pouch like that in Figure 4 but considerably larger.

FIG. 6. Encysted pouch. No orifice.

FIG. 7. Magnification of circled area of Figure 5. Note normal epidermal lining and complete absence of inflammatory exudate.

FIG. 8. Magnification of circled area of Figure 6. Note connective tissue lining around nest. Depth of penetration of inflammatory cells around periphery very clearly delineated.

lateral borders, but compressed along the fundus of the pouch which was deep in the sub-cutis. At this stage, the opening was quite large and the mites could freely migrate or be accidently expressed out of the nest.

An encysted nest is shown in Figure 6. The lumen was lined with a layer of connective tissue and no trace of epidermis remained. The cyst had no orifice and a marked invasion by inflammatory cells was in progress. Figures 7 and 8 are enlargements of a portion of the normal pouch in Figure 5 and the cyst in Figure 6, respectively. In the former, the epidermal lining was intact and normal and no inflammation was seen. In the latter, the epidermis had been replaced by connective tissue and inflammatory cells had penetrated well into the cyst.

DISCUSSION

Piana (1886) believed that pouch formation was initiated by penetration by mites through the epidermis; as the pouch increased in size the derma was displaced and stretched into a very thin layer. We believe that the typical pouch is initiated by the entrance of one or more parasites into a hair follicle as proposed by Neumann (1893). The continuity of the epidermis around the pouch (Figs. 3, 4, 5) supports this suggestion. The normal thickness and overall appearance of the epidermis (Fig. 7) suggests that enlargement of the pouch is through growth of the epidermis adjusting to the internal pressures of the pouch. The cyst (Fig. 6) with its content of inflammatory cells is probably an immune response to the infestation; perhaps induced by parenteral introduction of the mites from a ruptured pouch.

The life cycle of the mite apparently is completed in the nest as indicated by the presence of all stages of the cycle in any fair-sized pouch. They may feed on the detritus in the nest which appears to be cornified epidermis which has sloughed off the walls of the pouch. The relatively large size of the opening in the typical nest (Fig. 5) permits ready escape either by chance movements or accidental expulsion. Colonization may occur as the result of the entry of a wandering gravid female into another hair follicle. It is likely that exchange of hosts takes place through contact.

SUMMARY

Nest formation by *Psorergates simplex* in the skin of mice is apparently initiated by the entry into a hair follicle of one or more individuals. The resultant pouch is an invagination of the skin lined with epidermis and opening to the outside. It contains several generations of mites in all stages of development. The growth of the pouch does not usually incite an immune response, but when it does, the nest becomes encysted and invaded by inflammatory exudate.

ACKNOWLEDGMENT

We wish to express our appreciation to Miss J. K. Glaser and Mr. A. S. Tracy for the photomicrography.

LITERATURE CITED

CARTER, H. B. 1941 A sk n disease of sheep due to an ectoparasitic mite, *Psorergates ovis* Womersley 1941. Austral. Vet. J. **17:** 193–201.

NEUMANN, L. G. 1893 Sur un acarien (*Psorergates simplex* Tyrrell) de la souris. Bull. soc. hist. nat., Toulouse. **27:** 13–21.

PIANA, G. P. 1886 Cisti cutanee contenenti acari nei topi. Annu. R. Scuola med. super. Milano med. vet., **1:** 122. (Cited by Neumann, 1893).

SAMBON, L. W. 1928 *Psorergates simplex* as a possible factor in epithelioma of the skin and mammary glands in murids. Ann. Trop. Med. and Parasitol. **22:** 133–136.

A NEW SPECIES OF *COELOMOMYCES* (FUNGI) FROM TASMANIAN MOSQUITO LARVAE

MARSHALL LAIRD
Department of Parasitology, University of Malaya, Singapore

Coelomomyces Keilin is referable to the family COELOMOMYCETACEAE Couch of the fungal order BLASTOCLADIALES (Couch, 1945). All the species are obligate parasites of insect larvae and/or adults, and with but a single known exception—*C. notonectae* (Bogoyavlensky)—they parasitize mosquitoes.

The study of these interesting organisms has, until recently, been neglected in Australasia. Descriptions of new species from Queensland and the Solomon Islands, and new locality records for previously known species, are at present in press (Laird: *Royal Society of New Zealand*). The material considered herein was kindly made available by Dr. Elizabeth N. Marks of the Department of Entomology, University of Queensland, who collected it in Tasmania.

MATERIAL AND METHODS

Parasitized larvae of *Aëdes* (*Pseudoskusea*) *australis* (Erichson) were dipped from a brackish rock pool on the ocean beach north of Davey Head near Trumpeter Island, Port Davey, Tasmania, on January 30, 1954. This description is based on material from a single fourth instar larva, which appeared of a golden-brown color to the unaided eye. The head capsule and thorax proved to be packed with mature sporangia, which were plentiful in the haemocoele of the anterior abdominal segments and present, but rare, in that of the siphon, saddle and anal papillae.

Pieces of the thorax and abdomen were teased out with needles, passed through the alcohol-xylol series, and mounted unstained in Stafford Allens' "Sira" medium. The observations were made by phase-contrast illumination, the figures being prepared by means of a Zeiss-Winkel drawing apparatus.

DESCRIPTIVE ACCOUNT

(All measurements in microns and all averages computed from the measurement of 100 examples)

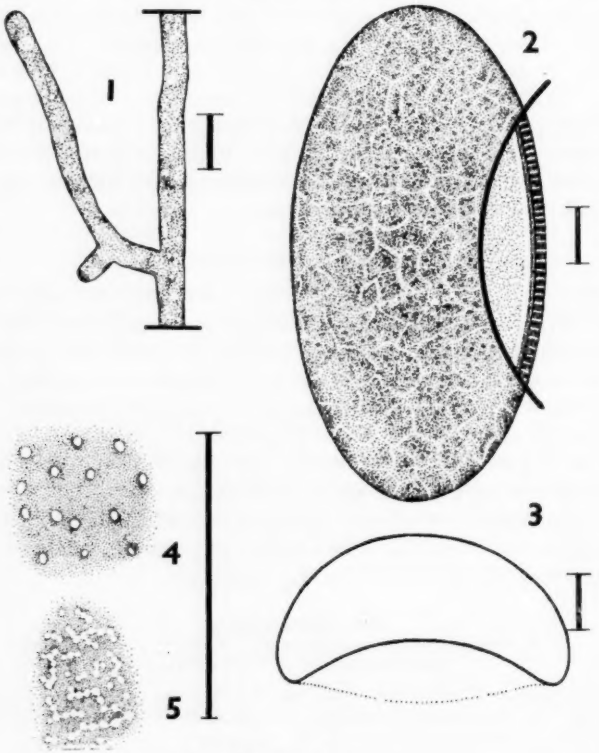
Hyphae. Mycelium characteristic of the genus, being coenocytic, aseptate and without cell walls. Hyphae (Fig. 1) abundant, branching freely although seldom anastomosing, and ranging from 3.0 to 6.0 in thickness (av., 4.8).

Sporangia. Elongate oval in surface view (Fig. 2), one face strongly convex and the other flat or else sufficiently concave for the sporangium to appear kidney-shaped in end view (Fig. 3) and boat-shaped in lateral view. Mature sporangia usually about twice as long as broad, ranging from 79.3 to 109.5 in length (av., 93.4) and from 36.5 to 56.6 in breadth (av., 47.6). Wall two-layered, and of a total thickness of from 2.0 to 4.0 (av., 2.8). The inner layer smooth and hyaline, averaging 0.6 in thickness and thus only one-quarter to one-fifth the thickness of the golden-brown outer wall. The latter pierced by great numbers of tiny, closely set pits, by virtue of which it appears as if striated in sectional view (Fig. 2, inset). These pits are round or oval in shape and range from 0.3 to 0.5 in diameter in mature sporangia in which they are usually set a little over 1.0 apart from one another (Fig. 4). Immature sporangia have smaller but often more numerous pits, which seldom exceed 0.3 in diameter and frequently anastomose (Fig. 5). The outer wall, although smooth, appears to bear a reticular ornamentation (Fig. 2) which shows brilliantly by phase contrast and quite distinctly by ordinary bright field illumination. This is due to plasmolysis inducing crenation of the surface of the enclosed protoplasmic mass which shows through the translucent walls.

Received for publication, September 9, 1955.

SYSTEMATIC POSITION

Mature sporangia of the *Coelomomyces* under consideration are very much larger than those of any other of the known species of the genus with the exception of *C. psorophorae* Couch, 1945. The latter parasite was described from a single larva of *Psorophora ciliata* (Fabr.) from Georgia, and remains known only from the U.S.A. It has since been recorded from *Aëdes vexans* Meigen in Georgia, from *Psorophora howardi* Coquillet in South Carolina and from *Culiseta inornata* (Willis-



KEY TO THE FIGURES

(The line on the right-hand side of each figure represents ten microns at the same magnification)

FIG. 1. A branched hypha. ×850. FIG. 2. Mature sporangium, surface view; inset, sectional view. ×850. FIG. 3. End view of sporangium, diagrammatic. ×850. FIGS. 4 and 5. Detail of surface of outer wall showing pitting in mature and immature sporangia respectively. ×4,350.

ton) in Louisiana (Couch and Dodge, 1947). Hyphae were not well developed in the type collection (Couch, 1945), although later batches of material displayed abundant mycelium (Couch and Dodge, 1947). However, the hyphae of the Tasmanian parasite are more slender than those of *C. psorophorae*, ranging from 3.0 to 6.0 while those of the latter species measure from 7.5 to 10.0 (Couch, 1945).

The sporangial wall of *C. psorophorae* ranged from 3.0 to 10.0 in thickness in

the type material, but in the examples from *A. vexans* it did not exceed 5.5 in thickness and in those from *C. inornata* it only reached 5.0 (Couch and Dodge, 1947). The outer wall is pierced by "vast numbers of minute anastomosing pits" (Couch, 1945) located less than 1.5 from one another (Couch and Dodge, 1947), but the sporangium is not otherwise ornamented.

Overall measurements of *C. psorophorae* sporangia are as follows: 46 to 100 by 37 to 67 (*P. ciliata*) (Couch, 1945), 63 to 94 by 42 to 66 (*A. vexans*) (Couch and Dodge, 1947), up to 119 by 58 (*C. inornata*) (Couch and Dodge, 1947). While the length thus reaches lower and higher extremes than does that of the parasite of *Aëdes australis,* both the usual size and the length: breadth ratio of mature sporangia appear much the same in the two organisms.

There are obviously close affinities between *C. psorophorae* and the species under consideration. The much greater thickness attained by the sporangial wall of the former parasite need not necessarily be of specific significance, as is indicated by the fact that Couch and Dodge (1947) did not record any sporangia in which the wall exceeded 5.5 in thickness from *A. vexans* or *C. inornata.* Of more importance is the fact that while the mature sporangium of *C. psorophorae* is frequently slightly flattened on one side (Couch, 1945) that described herein always has one side either flat or concave. Because of this, the other sporangial discrepancies and the facts that the Tasmanian fungus has much more slender hyphae and infects larvae occurring in a brackish habitat as opposed to freshwater ones, the latter parasite is considered to be distinct and is accordingly designated *Coelomomyces tasmaniensis* n. sp.

A type slide has been lodged in the collection of the Dominion Museum, Wellington, New Zealand, and a paratype has been deposited in that of the Department of Entomology, University of Queensland, Brisbane, Australia.

Literature Cited

Couch, J. N. 1945 Revision of the genus Coelomomyces, parasitic in insect larvae. J. Elisha Mitch. Sci. Soc. **61:** 124–136.

——— and Dodge, H. R. 1947 Further observations on Coelomomyces, parasitic on mosquito larvae. J. Elisha Mitch. Sci. Soc. **63:** 69–79.

PROBOLITREMA MEXICANA, N. SP., AN ANAPORRHUTINE TREMATODE FROM ELASMOBRANCHS OF BAJA CALIFORNIA

EDWARD K. MARKELL

Department of Infectious Diseases, School of Medicine,
University of California, Los Angeles

Four trematodes, found in the body cavities of elasmobranch fishes from Santa Inez Bay, on the Gulf of California, Baja California, were kindly turned over to me for identification by Dr. Clark P. Read. Two were from *Urobatus maculatus*, one from *Dasyatis brevis*, and one from *Mustelus lunulatus*. The worms had been fixed in Bouin's fluid, and were stained with borax-carmine. They were found to belong to a new species of *Probolitrema*, which is here described as *Probolitrema mexicana*.*

Probolitrema mexicana, n. sp.

Specific diagnosis: With the characters of the genus *Probolitrema*. Sexually mature specimens 3.6 to 17.5 mm long, and from 2.6 to 14.0 mm in greatest width. Ratio of oral to ventral sucker, 2:3. Intestinal ceca finely diverticulate to grossly saccate. Testes extracecal; from 17 to 39 lobate follicles in each testis. Vas deferens coiled, expanded anteriorly into large muscular seminal vesicle and smaller pars prostatica. Ovary deeply lobate; three to five lobes. Vitellaria with irregularly nodular tubular branching processes. Ova 50–66 by 34–40 microns.

Type host: Mustelus lunulatus.
Other hosts: Urobatus maculatus, Dasyatis brevis.
Habitat: Coelomic cavity.
Locality: Santa Inez Bay, Baja California, Mexico.
Type specimen: U. S. Nat. Mus. Helm. Coll., No. 49354.

DESCRIPTION

The worms are irregularly oval, their greatest width being approximately equidistant from the anterior and posterior ends. The posterior end is usually rounded, with an indentation in the midline marking the site of the excretory pore; the anterior end may narrow abruptly forming a definite neck, or taper uniformly to the oral sucker.

Measurements given hereafter, unless otherwise noted, are of the type specimen (Fig. 1), a worm 13.7 mm in length. The oral sucker is terminal, and measures 1.12 mm across by 0.89 mm in length. The acetabulum is 1.46 mm in diameter, and is situated between one-quarter and one-third of the body length from the anterior end. The ratio between oral and ventral suckers seems quite constant, though the suckers are much larger in proportion to total body size in the smaller (and presumably younger) worms. The pharynx is nearly spherical, fitting closely against the posterior wall of the oral sucker, and measures 0.64 mm by 0.51 mm. The esophagus is thin-walled, with minute pointed diverticula coming off on all sides, and is 0.68 mm in length. The intestinal ceca, which like the esophagus have a fundamentally diverticulate structure, become grossly saccate when distended. The finer sharply pointed diverticula are not visible in the areas distended by food, but appear along the entire course of the ceca wherever they are distended. The ceca lie about half-way in toward the midline from the sides of the body.

Received for publication, July 12, 1954.

* Appreciation is expressed to Dr. George R. LaRue, who very kindly reviewed my specimens, and related forms in the Helminthological Collection of the National Museum, and made numerous helpful suggestions.

The testes are extracecal; each consists of 17 to 39 separate follicles. Each testicular mass occupies an area extending between one-half and three-fifths of the length of the body. The individual follicles are deeply lobed in the larger worms (Fig. 3, b), but unlobed or very simply lobed in the smaller ones (Fig. 3, a). The duct system uniting the follicles could not be traced in its entirety in any of the specimens, but enough could be seen to indicate that it is essentially the same as that found in other species of the genus: tubules from several follicles unite, the tubules thus formed unite with similar ones, and finally the collecting tubules come together to form the vas deferens. The vasa efferentia can be traced to the midline, where they unite anterior to the ventral sucker to form a vas deferens. The posterior portion of the vas deferens, coiled upon itself, is approximately 0.13 mm in width, and expands anteriorly into a muscular seminal vesicle, 1.02 mm long by 0.57 mm wide. From this, another coiled tubule, the pars prostatica, 0.21 mm in diameter and surrounded by a dense cluster of cells, leads to the genital pore. The genital pore is situated in the midline and posterior to the bifurcation of the intestine.

The three- to five-lobed ovary lies somewhat anterior to the middle of the body, and may be on either side of the midline. In the type specimen, it measures 0.81 mm in width by 0.59 mm in length. Anterior to the ovary, and partly dorsal to the ventral sucker lies the rounded thin-walled seminal receptacle, containing a mass of spermatozoa. Between the ovary and seminal receptacle run the vitelline ducts. The details of the connections between the vitelline ducts, oviduct, Mehlis' gland and the uterus cannot be made out, although Mehlis' gland is visible between the ovary and seminal receptacle. The vitellaria are extracecal, and may be compact, or quite loosely arranged. Their structure is tubular (Fig. 4) with one or several main stems, from which short or long irregularly nodular branches arise. The branches may anastomose, or branch secondarily. The uterus (Fig. 1) fills the area between the intestinal ceca posterior to the ovary, and runs on the antovarian side of the body up past the ventral sucker to open at the genital pore. The eggs become considerably enlarged during their passage through the uterus; those in the terminal portions measure 50–66 by 34–40 microns.

The excretory vesicle was poorly visualized, and could not be followed throughout its entire length in any of the specimens. Reconstruction of serial sections through one of the smaller worms indicates that the bladder runs anteriorly from the excretory pore, approximately in the midline, to bifurcate just posterior to the level of the ovary. In one worm, the posterior portion of the bladder wall could be clearly seen, and it was noted to have numerous short side-branches.

DISCUSSION

Seven species of *Probolitrema* have been previously described; their diagnostic characteristics are summarized by Markell (1953). *P. mexicana* differs from the other species of the genus in possessing diverticulate or saccate intestinal ceca. Johnston (1934) described small sacculations appearing posteriorly in the ceca of *P. rotundatum*, and these may also be seen occasionally in *P. californiense*, but are quite unlike the fine, pointed diverticula found in the undistended portions of the ceca of *P. mexicana*. Branching of the intestinal ceca is seen in the genera *Petalodistomum* as described by Johnston (1913) and *Nagmia* as described by Nagaty

(1930), although varying considera'ly in degree in the different species. These two genera differ from *Probolitrema,* however, in possessing vitellaria which are intracecal in position.

SUMMARY

A new species of anaporrhutine trematode, *Probolitrema mexicana,* is described from elasmobranchs of Santa Inez Bay, Baja California, Mexico.

REFERENCES CITED

JOHNSTON, S. J. 1913 On some Queensland trematodes, with anatomical observations and descriptions of new species and genera. Quart. J. Micr. Sc. **59:** 361–400.

JOHNSTON, T. H. 1934 Some Australian anaporrhutine trematodes. Tr. and Proc. Roy. Soc. South Australia **58:** 139–148.

MARKELL, E. K. 1953 Further observations on the anatomy of the gorgoderid trematode *Probolitrema californiense.* Trans. Amer. Micros. Soc. **72:** 68–77.

NAGATY, H. F. 1930 A new anaporrhutine trematode genus and species *Nagmia yorkei,* with a review of the classification of the sub-family. Ann. Trop. Med. and Parasitol. **24:** 97–108.

PLATE I

EXPLANATION OF FIGURES

FIG. 1. *Probolitrema mexicana,* whole mount, showing male and female reproductive systems.

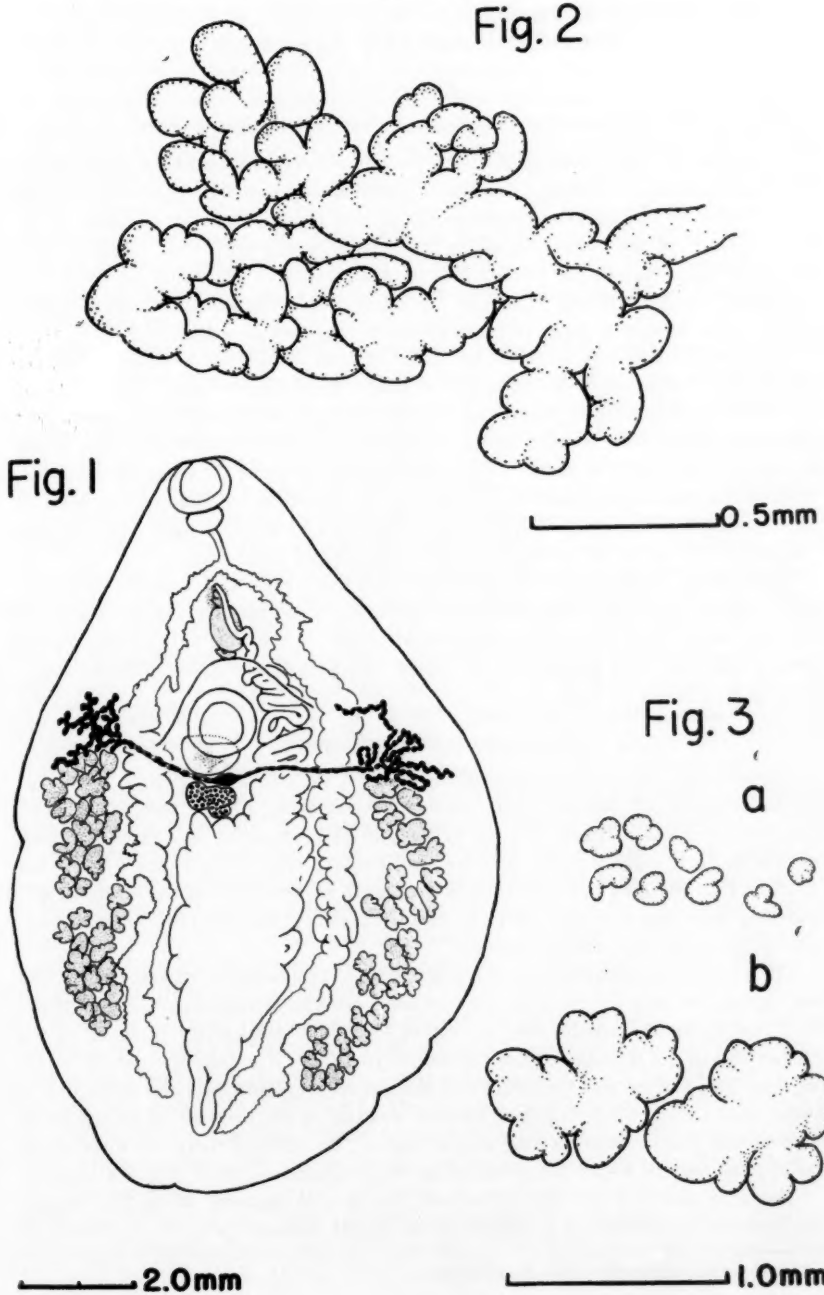
FIG. 2. Vitelline gland from a worm 17.5 mm in length.

FIG. 3, a. Testis follicles from a worm 3.6 mm in length.

FIG. 3, b. Testis follicles from a worm 17.5 mm in length.

All figures drawn with the aid of a camera lucida.

PLATE I



MYOCHERES INFLATA A NEW SPECIES OF PARASITIC COPEPOD FROM THE BAHAMAS

J. A. ALLEN

Dove Marine Laboratory, King's College, Newcastle-upon-Tyne

In the course of an investigation into the ciliary mechanisms of the LUCINACEA (EULAMELLIBRANCHIA) a new species of parasitic copepod has been found in the mantle cavity of *Lucina pennsylvanica* Linné and *Divaricella quadrisulcata* d'Orbigny. These species were collected in June, 1953 from Porgy Bay, North Bimini Island, during a visit to the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas. Both species of lamellibranch were found in clean coral sand at a depth of approximately four feet of water.

The parasite was not recorded by C. B. Wilson (1935) or Pearse (1951) in their works dealing with the parasitic crustacea from this region. The presence of a slender 6-segmented first antenna, the second segment of which is elongate, and a markedly prehensile second antenna, together with the characteristic form of the maxillae and the first four pairs of legs, would appear to place the parasite as a member of the family LICHOMOLGIDAE (Suborder CYCLOPOIDA). But, descriptions of related species by Yamaguti (1936), Pearse (1947) and M. S. Wilson (1950) make it necessary to reconsider whether a new family should be created.

The absence of maxillipeds in the female and the presence of a stout pair in the male, together with the characteristic triangular spines on the outer margins of both rami of the first pair of legs and the structure of the fifth pair of legs would appear to place it in the genus *Myicola* (see p. 62) first described by Wright (1885) and later by Wilson (1932).

Myocheres inflata, new species

Female. The animal is greyish white in color, the overall length (to the tip of the caudal setae) of the specimens collected varied from 1.8–3.7 mm. The head is heart-shaped and is fused with the first segment. Segments two, three and four are as wide as or slightly wider than the head and are very inflated (see Figs. 1 and 5). The fifth segment is less than half the length and two-thirds the width of the preceding segments yet, like them, is very inflated. This inflation is due to the exceedingly large uteri of the female.

The urosome is composed of four segments and tapers to the caudal rami. The first segment is long and exceeds the combined lengths of segments two and three and it carries the remarkably long curved egg sacs. The latter are attached to the anterior end of the segment in a dorso-lateral position. The fourth segment is also long but does not exceed the combined lengths of segments two and three. The caudal rami are the same length as the last segment of the urosome and each bears a caudal seta that is twice as long as the ramus. In addition there are three other caudal setae present which are arranged in the positions shown in Figures 1 and 5.

The first antennae are six segmented, the second segment being the longest. The setation is as shown in Figure 8. The second antenna (Fig. 7) is prehensile

Received for publication, May 20, 1955.

and three segmented. It bears one stout curved claw and three heavy curved setae which are slightly longer than the claw. These articulate with the apex of the distal segment. Laterally, this distal segment bears a short seta just below the articulation of the claw and is fringed with a row of short spines. In addition, there is a seta on the distal end of the first segment of the second antenna. The mouth parts consist of two pairs of appendages, the maxillipeds being absent. The nomenclature proposed by Monod and Dollfus (1932) is followed here. The labrum is simple and triangular in shape. The first maxilla (Figs. 2 and 3) is very complex and bears a setose 'flagellum,' a heavily toothed spine and a serrated shield-like structure. A relatively large palp is present that has three upwardly directed setae and one inwardly directed seta. The second maxilla is simple, having a swollen basal segment and a terminal segment that ends in a very heavy spine with one stout seta on the posterior side.

The first four pairs of legs (Figs. 4, 9 and 17) are but slightly modified. Both rami are composed of three segments, the outer bearing very heavy spines on each segment. The inner ramus has heavy spines on the terminal segment. The sternite of the first four pairs of legs forms a bridge connecting the inner rami and which bears a patch of spines at the base of each inner ramus. The first leg also carries a heavy spine between the patch of spines and the inner ramus. The first two segments of the outer ramus of the first leg bear characteristic triangular teeth. The second and third legs are identical and differ from the first in numbers of spines, teeth and setae. The fourth legs again differ slightly from the other legs and, like the first leg, have a slightly smaller number of spines and setae than the second and third legs. The fifth legs (Fig. 10) are two-segmented. There is a lateral seta on the proximal segment and also a number of terminal spines and setae. This leg is carried laterally on the fifth segment.

Male. The color of the male is similar to that of the female, the specimens collected were considerably shorter than the females, varying from 0.9–1.4 mm. in length. The head is similarly shaped to that of the female but the rest of the body shows distinct differences (Figs. 11 and 13). The body is not inflated but is dorsoventrally compressed and the segments gradually taper to the caudal region. The fifth body segment is almost as wide as the fourth and tends to be more rounded in outline than the preceding segments. There are five urosome segments, the first being almost as wide as the fifth thoracic but only half as long. The second urosome segment is stout, short but not noticeably swollen. The fifth segment is the longest and has a slight median groove on the dorsal side. The caudal rami and their setation resemble those of the female.

The first antenna is very similar to that of the female with the second segment the longest (Fig. 12). It is six segmented with minor differences in the setation. The second antenna is also similar to that of the female and apart from slight differences in the shape of the segments, the only major difference is in the possession of a patch of spines on the second segment (Fig. 15). The first maxilla shows little difference from that of the female, while the second maxilla differs only in that the male has a small patch of spines on the basal segment (Fig. 14). The male possesses typically large prehensile maxillipeds (Fig. 16). The proximal segment of the maxillipeds carries a large seta on the distal end near the joint while the distal segment carries a very large terminal claw which bears a seta at its base.

Laterally on the shoulder of the distal segment there is a small patch of spines. The legs are identical to those of the female.

Hosts: *Lucina pennsylvanica* Linné and *Divaricella quadrisulcata* d'Orbigny, mantle cavity.

Locality: Porgy Bay, North Bimini Island, Bahamas.

Type Specimens: Dove Marine Laboratory, King's College, Newcastle-upon-Tyne and British Museum (Natural History), London.

DISCUSSION

The parasite was present in approximately 70% of the specimens of *Lucina pennsylvanica* examined. They were present to a much lesser extent in *Divaricella quadrisulcata* where they were in 10% of the specimens examined. This emphasizes the fact that although these cyclopoid parasites show a marked preference for one host they can and will parasitize other species. However, *Myocheres inflata* is of interest in that it appears to be well adapted to the feeding methods of the LUCINACEA. The LUCINACEA are unusual in that they build an anterior inhalent tube and this results in a new type of feeding mechanism (Allen 1953 and unpublished work.) The anterior adductor muscle is elongate and ciliated for the transport of food particles to the mouth and the female parasite invariably was found curled round the ventral end of the muscle. The head of the parasite was lying towards the mouth of the lamellibranch and the whole animal lay over the ciliated tract. Never more than one parasite was found in each mollusc. The males were never found on the anterior adductor muscle but were always in the general mantle cavity. Only 20% of the animals taken were males and it seems possible that while the females remain in the one host the males are not so restricted.

Myocheres inflata differs from *Myocheres major*, a closely related species (see below), in that the males found were much smaller than the females. It is possible that either fully grown males were not collected, or in this species the males are necessarily small in order to pass down the inhalent tubes of the young lamellibranchs. Mature female parasites were found in lamellibranchs less than 1 cm. in length and a specimen of this size would build a tube of approximately 0.5 mm. diameter. No deleterious effect on the host was observed.

The new species described above shows great resemblances to a parasite found in various lamellibranchs at Rhode Island by Williams (1907) and named by him *Lichomolgus major*. C. B. Wilson (1932) considered that this species was sufficiently like *Myicola metisiensis* Wright, 1885 to be included in the same genus. M. S. Wilson (1950) re-examined *Lichomolgus major* and concluded that it differed sufficiently from *Myicola* to create the genus *Myocheres*. The great similarity between the new species and *M. major* gives further emphasis to the large number of differences between the latter and *Myicola metisiensis*.

The main differences between *Myocheres inflata* and *M. major* are that the former has only one claw on the second antenna instead of two; *M. major* has considerably longer fifth legs; various differences in shape and sizes of the sexes, particularly the males of the two species, and various differences in numbers of spines and setae. M. S. Wilson in her generic diagnosis states that *Myocheres* is not inflated; this statement must now be modified with the discovery of the new species, the appearance of which gives rise to the specific name *inflata*.

M. S. Wilson points out that other species have been erroneously ascribed to the genus *Myicola* and has established that *Myicola spinosa* Pearse is synonymous with *Myocheres major*. Pearse (1947) has also described a closely related species *Myicola tageli* and *Pseudomyicola glabra*. The latter genus was first described by Yamaguti (1936) who considers it to be closely related to *Myicola*. At the same time, but without giving reasons or diagnosis, Yamaguti created a new family the MYICOLIDAE to include the above two genera.

The descriptions of the known species of *Myicola*, *Pseudomyicola* and *Myocheres* when compared show that *Myicola metisiensis* differs considerably from the other related species (see Table I), while the genera *Myocheres* and *Pseudomyicola* are

TABLE I.—*A comparison of the known species of the family* Myicolidae

	Body proportions thorax : abdomen		1st antenna	2nd antenna	Maxilla	Legs 1–4	Leg 5
	male	female					
Myocheres major and *M. inflata*	9 : 7	12 : 11	6-segmented, 2nd segment the longest	3-segmented, 1 or 2 claws, 3 heavy spines	*	**	2-segmented
Myicola tageli	2 : 1	2 : 1	6-segmented, 1st segment the longest	3-segmented, 1 claw, no spines	*	**	2-segmented
Myicola metisiensis	8 : 5	8 : 3	7-segmented, 1st, 2nd and 5th longer than others	4-segmented, 1 claw, no spines	*	**	3-segmented
Pseudomyicola glabra	2 : 1	2 : 1	6-segmented, 1st segment longest	3-segmented, 1 claw, no spines	*	**	2-segmented
Pseudomyicola ostreae	—	5 : 3	6-segmented, 1st longest	3-segmented, 1 claw, 1 basal and 3 terminal setae	*	**	2-segmented

* Marked differences between the species see Figs. 2 and 3, Fig. 9 Wright (1885), Fig. 5 Yamaguti (1936).

** Differences in numbers of spines and setae of these species.

also clearly defined. However, *Myicola tageli* Pearse appears to resemble *Myocheres* more than it does *Myicola* and it is probable that re-examination of the types would confirm that it should be included in the former genus. The removal of the above three genera from the LICHOMOLGIDAE and including them in the separate family MYICOLIDAE appears to be justified for the following reasons: that only *Myicola metisiensis* has a 7-segmented first antenna the other five species all have a 6-segmented antenna, furthemore the second segment is not necessarily the longest as is true in the LICHOMOLGIDAE; the anterior lip, where described, is not deeply cleft and the fifth leg is two or three segmented and never one segmented as in the LICHOMOLGIDAE. The MYICOLIDAE appear to be found only as lamellibranch parasites.

Key to the known species of the family MYICOLIDAE

1. First antenna 7-segmented, 5th leg 3-segmented ..... *Myicola metisiensis*
   First antenna 6-segmented, 5th leg 2 segmented 2
2. Heavy conspicuous spine at base of first antenna 3
   No conspicuous spine at base of first antenna 4
3. Second antenna without setae ..... *Pseudomyicola glabra*
   Second antenna with 1 basal and 3 terminal setae ..... *Pseudomyicola ostreae*
4. First antenna with the first segment the longest ..... *Myicola tageli*
   First antenna with the second segment the longest 5
5. Second antenna with two claws ..... *Myocheres major*
   Second antenna with one claw ..... *Myocheres inflata*

I am indebted to Dr. C. M. Breder Jr., and the Staff of the Lerner Marine Laboratory for their kindness and help and, to the John Murray Committee of the Royal Society for a grant that made this work possible.

SUMMARY

Myocheres inflata, a new species of cyclopoid copepod, is described. The genera *Myicola* Wright, *Pseudomyicola* Yamaguti and *Myocheres* have been compared, the erection of the new family MYICOLIDAE by Yamaguti (1936) has been upheld, and a key to the known species given.

REFERENCES CITED

ALLEN, J. A. 1953 Function of the foot in the Lucinacea (Eulamellibranchia). Nature **171:** 1117.

MONOD, T. AND DOLLFUS, R. P. 1932 Les copépodes parasites de mollusques. Ann. Parasitol. **10:** 129–204.

PEARSE, A. S. 1947 Parasitic copepods from Beaufort, North Carolina. Jour. Elisha Mitchell Sci. Soc. **63:** 1–16.

———1951 Parasitic crustacea from Bimini, Bahamas. Proc. U. S. Nat. Mus. (3280) **101:** 341–372.

WILLIAMS, L. W. 1907 A list of the Rhode Island Copepoda, Phyllopoda, and Ostracoda with new species of Copepoda. 37th. Ann. Rep. Comm. Inland Fisheries Rhode Island. 69–80.

WILSON, C. B. 1932 The copepods of the Woods Hole region Massachusetts. Bull. 158, U. S. Nat. Mus. xix–635 pp.

———1935 Parasitic copepods from the dry Tortugas. Pap. Tortugas Lab. **29:** 327–347.

WILSON, M. S. 1950 A new genus proposed for *Lichomolgus major* Williams (Copepoda, Cyclopoida). Jour. Wash. Acad. Sci. **40:** 298–299.

WRIGHT, R. R. 1885 On a parasitic copepod of the clam. Am. Nat. **19:** 118–124.

YAMAGUTI, S. 1936 Parasitic copepods from Mollusks of Japan, I. Jap. J. Zool. **7:** 113–127.

Myocheres inflata. All measurements are in millimeters.

PLATE I

1. Dorsal view of the female with egg sacs.
2. Maxilla 1—male and female.
3. Maxilla 1 with palp attached—male and female.
4. Second and third leg—male and female.

PLATE II

5. Lateral view of the female.
6. Maxilla 2—female.
7. Antenna 2—female.
8. Antenna 1—female.
9. Fourth leg—male and female.
10. Fifth leg—male and female

PLATE III

11. Lateral view of male.
12. Antenna 1—male.
13. Dorsal view of male.
14. Maxilla 2—male.
15. Antenna 2—male.
16. Maxillped—male.
17. First leg—male and female.

PLATE I

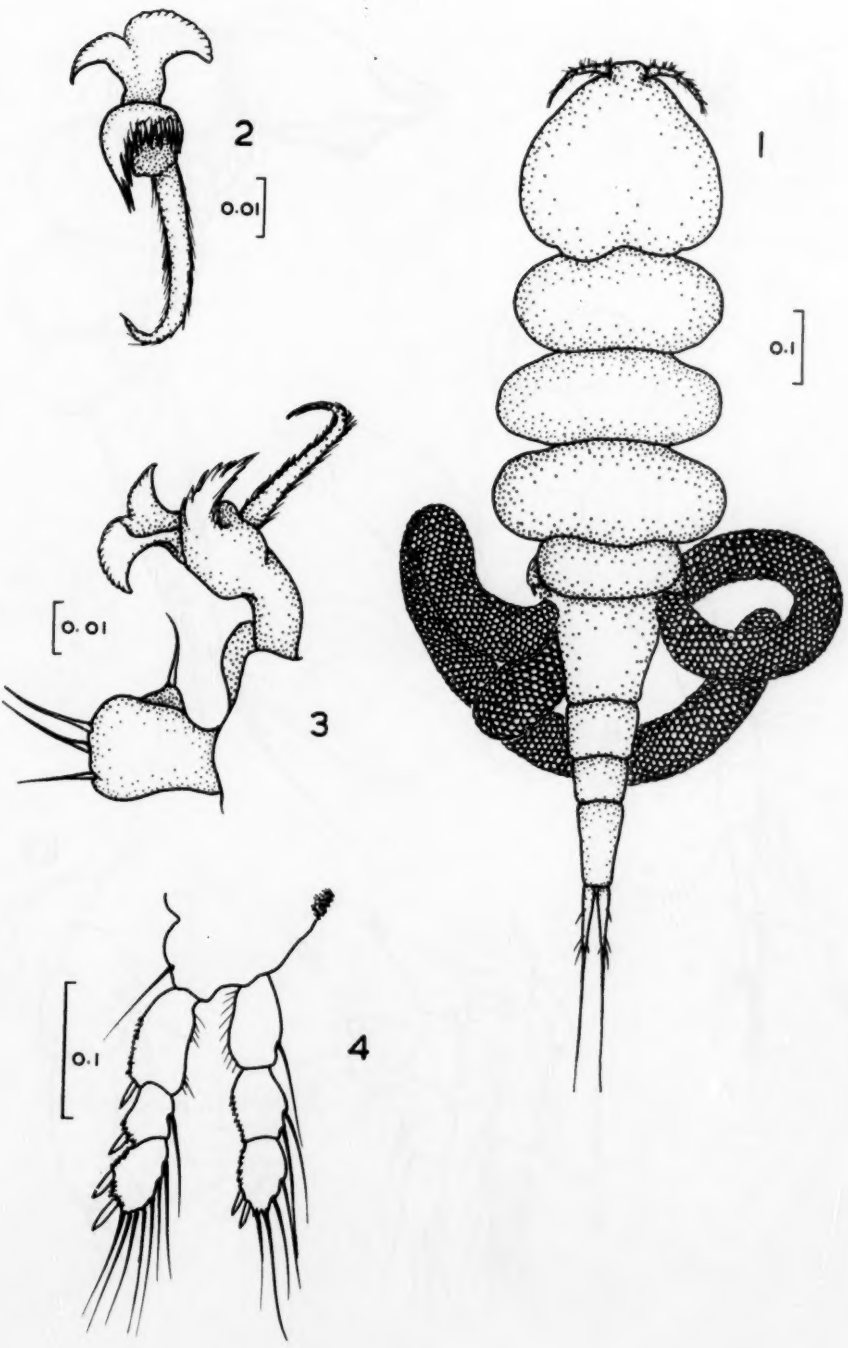


PLATE II

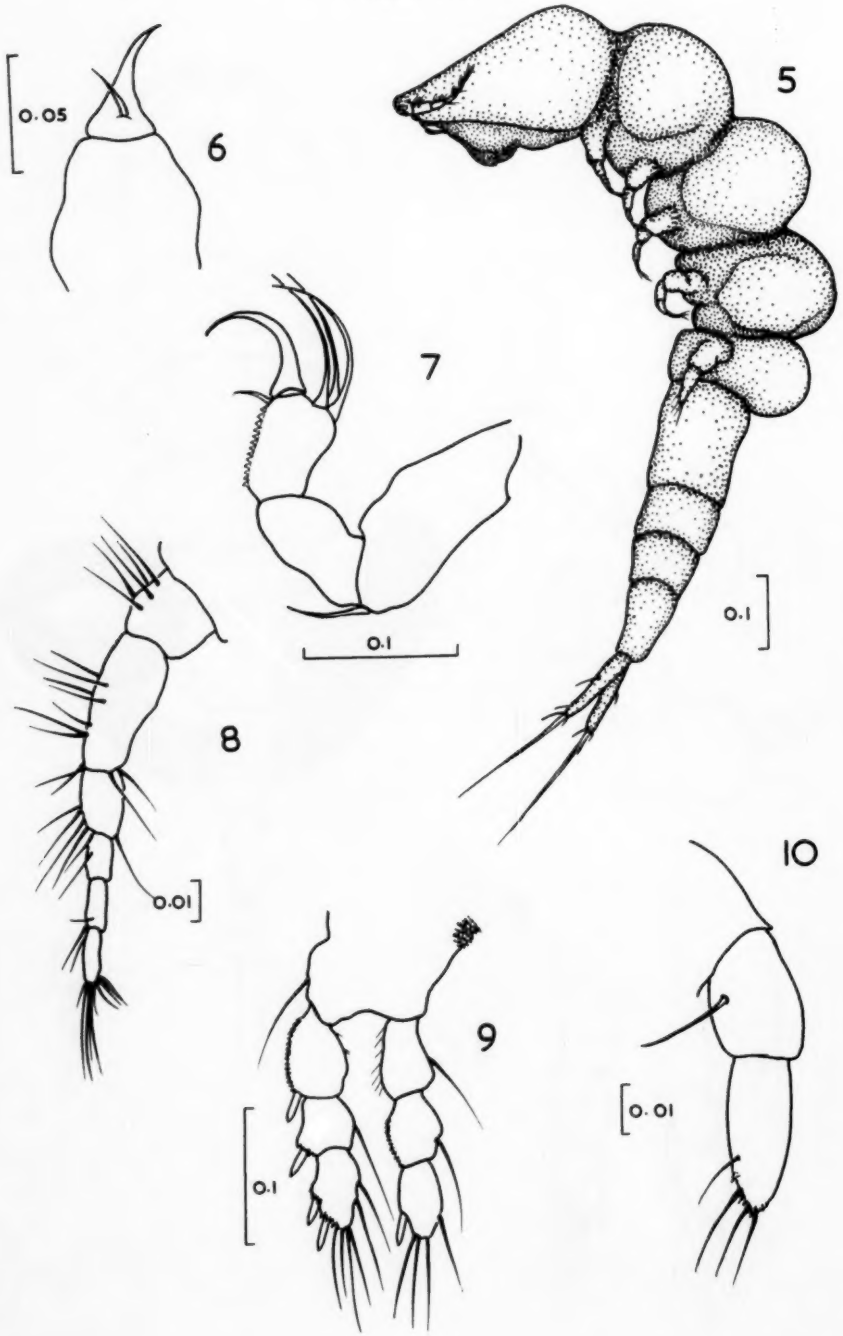
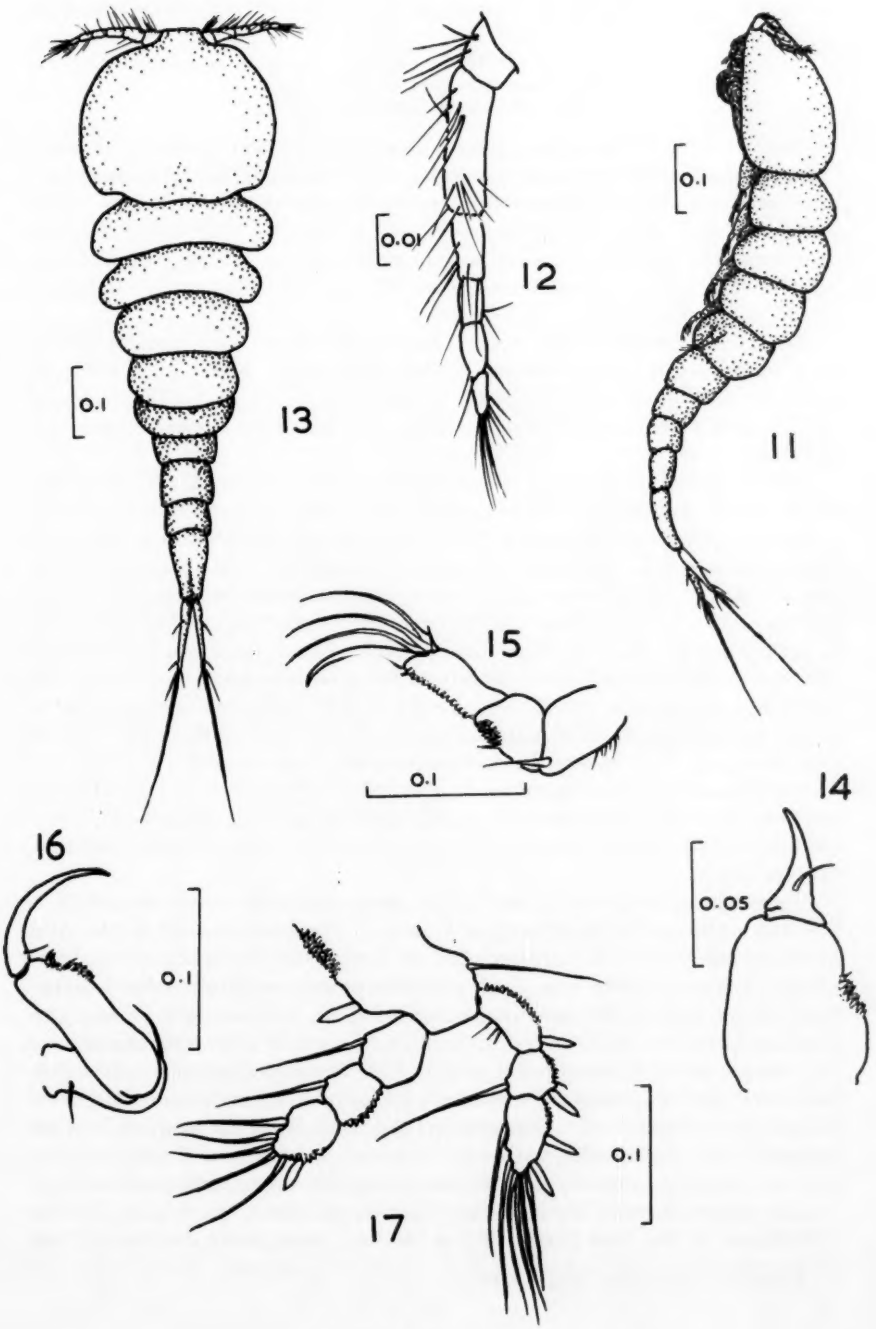


PLATE III



A REDESCRIPTION OF *CRUZIA AMERICANA*, A NEMATODE PARASITIC IN THE OPOSSUM, *DIDELPHIS MARSUPIALIS VIRGINIANA*

JOHN L. CRITES
Department of Zoology and Entomology
Ohio State University

Thirty-two of 44 opossums collected from Franklin and Delaware Counties, Ohio, harbored large numbers of nematodes of the genus *Cruzia* in the caecum and large intestine. The helminths were alive when removed from the hosts. Both warm AFA and warm 70% alcohol were used as fixatives. The nematodes were cleared in glycerine alcohol and in lacto-phenol. Specimens were examined with both ordinary light and phase microscopes. All measurements are from temporary mounts of specimens and are in millimeters.

The family CRUZIIDAE and genus *Cruzia* were erected by Travassos (1917) for a single species, *Ascaris tentaculata,* Rudolphi, 1819. Khalil (1927), Maplestone (1930), Khalil and Vogelsang (1930, 1932), Harwood (1932), Pearse (1936), Ruiz (1947), and Wolfgang (1951) have raised the number of species of this genus to twelve.

Only 3 species of *Cruzia* have been reported from marsupials: *C. tentaculata* (Rud. 1819) Travassos, 1917 from *Didelphis aurita* from South and Central America, *C. americana* Maplestone, 1930 from *Didelphis marsupialis virginiana* in Pennsylvania, and *C. cameroni* Wolfgang, 1951 from *Didelphis marsupialis insularis* in Trinidad. Maplestone (1930), following a suggestion by Travassos (1922) that the species of *Cruzia* from North American opossum might de different from *C. tentaculata,* proposed the name *Cruzia americana* for specimens that Canavan (1929) had identified as *C. tentaculata* from *Didelphis marsupialis virginiana.* The author has attempted to obtain these specimens of *C. americana* and has been informed by both Dr. W. P. N. Canavan and by Dr. H. L. Ratcliffe of the Philadelphia Zoological Gardens that the specimens which Canavan (1929) described are no longer available. The description by Canavan (1929) is brief and contains only one illustration, the tail of the male, lateral view. Since there is no description of the lips or other anterior structures, it is impossible to compare them with those of other species.

The author presented male and female specimens of the nematodes which he collected in Ohio to the U. S. National Museum. These were studied by Mr. Allen McIntosh and Mrs. M. B. Chitwood, and compared with other specimens of *Cruzia* which had been recovered from the North American opossum from other localities. The major consistent differences were due mostly to fixation. The author, after checking specimens in U. S. N. M. Helm. Coll., became convinced that there is only one species of *Cruzia* present in the North American opossum. As will be demonstrated, *C. americana* of the North American opossum can be shown to be morphologically different from *C. tentaculata* of the South American opossum, and also different from other species of *Cruzia.* Since all specimens of *Cruzia* examined from the North American opossum are morphologically similar, and therefore represent one species, it seems highly probably that the specimens described by Canavan (1929) were of this same phenotype, but had been incompletely described by him.

Received for publication, Aug. 9, 1955.

Since the species *Cruzia americana* Maplestone, 1930 was based on this description, and since Canavan's specimens are no longer available as types, the author has filed specimens collected in Ohio as Neotypes of *Cruzia americana*, and the following redescription of this species is given:

Cruzia americana Maplestone, 1930
Figures 1–13
(Measurements in millimeters)

Diagnosis: CRUZIIDAE (Travassos, 1917). Worms straight, fairly thick, tapering gradually distally. Greatest diameter between mid-point and esophogeal bulb. Cuticle smooth and thick. Mouth surrounded by three triangular lips. Dorsal lip, one pair of papillae on inner margin, two double papillae on outer margin. Sub-ventral lips one pair of papillae on inner margin, one double papilla on outer margin, and one prominent amphid. Lips 0.054 to 0.072 in depth. Cervical papillae, one pair, 1.05 from anterior end.

Male: Mature males 11.1 to 12.3 in length by 0.49 to 0.64 in width. Entire esophagus 2.11 to 3.14 long. Esophagus divided into four parts: First part, pharynx, 0.16 to 0.22 long, with 15 to 18 tooth-like projections directed inward; second, tubular part, 1.59 to 1.75 long: third part, anterior bulb, 0.09 to 0.11 long; fourth part, posterior bulb, muscular, valvular, 0.27 to 0.30 long. Intestinal diverticulum, 0.81 to 0.98 long. Nerve ring 0.44 to 0.61 and excretory pore 1.11 to 1.94 from anterior end, respectively. Distance anus to tip of tail, 0.17 to 0.22. Two spicules, alate, 0.89 to 1.00 long. Gubernaculum, 0.14 to 0.16 in length. Male tail pointed, curved slightly ventrad, bearing one median ventral pre-cloacal papilla and 11 pairs of papillae: Three pairs pre-cloacal, some distance apart; three pairs ad-cloacal, lying close together; five pairs post-cloacal, arranged as three ventral pairs and two lateral pairs.

Female: Mature females 16.4 to 18.3 long and 0.63 to 0.67 wide. Esophagus 2.23 to 2.41 long: Pharynx 0.16 to 0.18 in length, tubular portion 1.69 to 1.81 long, anterior bulb 0.08 to 0.09 long, posterior bulb 0.27 to 0.32 long. Intestinal diverticulum 0.88 to 1.22 long. Nerve ring 0.49 to 0.55 and excretory pore 1.25 to 1.57 from anterior end, respectively. Vulva transverse, slightly anterior to middle of body, lips protruding. Vagina muscular, 0.59 to 0.62 long, two vaginal glands. Ovijector, a triangular muscular sac, 0.58 to 0.70 in length. Vaginae uterinae, two, 0.29 to 0.30 long. Uteri, two, divergent, almost straight, 2.69 to 3.08 long. Oviducts, two, 7.20 to 11.00 long, coiled near ovaries. Ovaries, two, 10.20 to 13.01 long, diameter decreasing distally, each terminating in cap cell. Ova 0.10 to 0.12 long by 0.05 to 0.07 wide, oval and rugose. Female tail 0.73 to 0.98 long.

Host: Didelphis marsupialis virginiana Kerr, 1792.

Type specimens: Neotype (male) and allotype (female), in Helminthological Collection of U. S. National Museum, No. 38101; paratypes in the Museum of Parasitology, Ohio State University Dept. of Zoology and Entomology.

Site of Infection: Caecum and large intestine.

Locality: Franklin and Delaware Counties, Ohio.

DISCUSSION

Cruzia americana has eleven pairs of caudal papillae; three pre-cloacal, three ad-cloacal and five post-cloacal pairs; the post-cloacals are arranged as two lateral and three ventral pairs (Figs. 11, 13). The number and arrangement have remained constant in all specimens recovered from both natural and experimental infections. Harwood (1932) describes *Cruzia testudinis* from the turtle, *Terrapene carolina triunguis*, with nine pairs of caudal papillae; however an examination of specimens of this species shows that it also has eleven pairs of caudal papillae. *C. testudinis* may be distinguished from *C. americana* in the length of the female tail, length of the pharynx and length of the gubernaculum. *C. mexicana*, reported by Khalil (1927) from a mexican lizard, also has five pairs of post-cloacal papillae. It is readily distinguished from *C. americana* in having only two pairs of pre-cloacal papillae, equal spicules and a shorter gubernaculum. In *C. mexicana* the ratio of the female tail to the length of the body is 1 : 16, while in *C. americana* this ratio is 1 : 20 to 1 : 23. Travassos (1922) described *C. tentaculata* with nine pairs of

caudal papillae: three pre-cloacal, three ad-cloacal and three post-cloacal pairs. Ruiz (1947) described *C. tentaculata* with three pre-cloacal, three ad-cloacal and four post-cloacal pairs of papillae. The author examined six male specimens of *C. tentaculata* from *Didelphis aurita* collected in Brazil, U. S. Nat. Mus. Helm. Coll. No. 32538. These specimens have four post-cloacal pairs of papillae. In this, and other characteristics, they agree with the description of Ruiz (1947). *C. cameroni* also has ten pairs of caudal papillae with the pairs arranged three, three, and four. However, Wolfgang (1951) notes that there is some variability of arrangement within the four post-cloacal pairs.

Other than the caudal papillae, *C. americana* differs from *C. tentaculata* in the following: *C. americana* has fourteen to eighteen tooth-like projections in the pharynx, generally sixteen, while *C. tentaculata* has eight to fourteen, generally ten. *C. americana* has cervical papillae; in *C. tentaculata* they are absent. Ruiz (1947) places stress on the branching of seminal glands and the gubernaculum in species identification. In *C. americana* the seminal glands have two main branches, each having three short ramifications (Fig. 7); in *C. tentaculata* the seminal glands have two main branches, each with five short ramifications. The gubernaculum of *C. americana* varies from *C. tentaculata* not only in size but in shape (Figs. 9, 10). When dissected from a specimen, the gubernaculum of *C. americana* shows a blunt terminal end. In *C. americana,* the ratio of the adult female tail to the length of the body is 1:20 to 1:23; in *C. tentaculata,* this ratio is 1:10.8 to 1:15. In *C. americana* the excretory pore is anterior to the cephalic end of the intestinal diverticulum (Fig. 1); in *C. tentaculata* the excretory pore is usually posterior to the tip of the intestinal diverticulum. A comparison of these two species shows that in *C. americana* the pharynx is longer, both esophageal bulbs are smaller, the gubernaculum is shorter, the male tail is shorter, not so rugose, and is less curved, and the lips of the vulva (Fig. 5) are more prominent.

Besides the number of caudal papillae, *C. americana* differs from *C. cameroni* in the following ways: The intestinal diverticulum of *C. cameroni* never reaches half way to the nerve ring; in *C. americana* it always reaches at least half way, usually farther. The excretory pore of *C. cameroni* is always nearer the nerve ring than it is to the anterior end of the intestinal diverticulum; in *C. americana* it is always nearer the diverticulum. The lips of the vulva of *C. cameroni* are non-salient. The female tail of *C. cameroni* is longer than that of *C. americana;* the ratio of the length of the female tail to the length of the body is 1:10 to 1:14. The spicules of *C. cameroni* which are equal, are longer than those of *C. americana* which are sub-equal. The male tail of *C. americana* is shorter and less curved.

Wolfgang (1951) points out the importance of cephalic structures in the separation of species of *Cruzia. C. americana* has two small single papillae toward the inner margin of each lip. The dorsal lip has two double papillae at the externo-lateral margins. The subventral lips each have one double papillae at the externo--lateral margin, and one prominent amphid. The lips, of *C. americana,* as seen in the *en face* view (Fig. 12), are somewhat similar to those of *C. testudinis* Harwood, 1932. *C. americana* differs from *C. cameroni,* as described by Wolfgang (1951), in that the latter species has no externo-lateral papillae on the subventral lips. Other papillae are the same although different in position. *C. tentaculata* differs from *C. americana* in that the papillae are different in position but the same in num-

ber. In *C. tentaculata,* the amphids are more medial on the subventral lips, and the outer papillae are toward the ventral side. Ruiz (1947) describes the papillae at the internal angle of the lips of *C. tentaculata* as being single, and double-branched. On specimens of *C. tentaculata* examined by the author, these appear as two small separate papillae, a character which appears to be constant for all the species examined. The subventral lips of *C. americana* protrude anteriorly beyond the dorsal lip (Fig. 4). The lips of *C. americana* are more arched and less triangular than those of *C. tentaculata;* they are not separated from the remainder of the body by obvious shoulders as are those of *C. cameroni.*

ACKNOWLEDGMENT

The author wishes to acknowledge a loan by Allen McIntosh of specimens of *Cruzia tentaculata* and *Cruzia* from four different localities in the United States. These specimens are located in the U. S. National Museum. The author also wishes to acknowledge the loan of specimens of *C. cameroni* by Robert W. Wolfgang and *C. testudinis* by Paul D. Harwood.

LITERATURE CITED

CANAVAN, W. P. N. 1929 Nematode parasites of vertebrates in the Philadelphia Zoological Gardens and vicinity. Parasitology **29:** 63–102.

HARWOOD, P. D. 1932 The helminths parasitic in the Amphibia and the Reptilia of Houston, Texas and vicinity. Proc. U. S. Nat. Mus. Art. 17, **81:** 1–71.

KHALIL, M. 1927 *Cruzia mexicana* n. sp. parasite d'un lezard mexicain. Ann. Parasitol. **5:** 41–45.

——— AND VOGELSANG, E. G. 1930 *Cruzia fülleborni,* a new species of nematoda from *Tupinambis teguixin.* Zentralbl. Bakt. Abt. I. Orig. **119:** 72–74.

——— AND VOGELSANG, E. G. 1932 On some nematode parasites from South American animals. Zentralbl. Bakt. Abt. I. Orig. **123:** 477–485.

MAPLESTONE, P. A. 1930 Nematode parasites of pigs in Bengal. Rec. Ind. Mus. Calcutta. **32:** 77–105.

PEARSE, A. S. 1936 Parasites from Yucatan. Carn. Inst. of Wash. Publication No. 457: 45–59.

RUIZ, J. M. 1947 Revisão do genero *Cruzia* (Nematoda: Oxiuroidea) e estudo das especies brasileiras. Thesis, S. Paulo: 1–105.

SPREHN, VON C. 1932 Über einige von Dr. Einsentraut in Bolivia gesammelte Nematoden. Zool. Anz. **100:** 273–284.

TRAVASSOS, L. P. 1917 Alguns helminthos da colleção do Instituto Bacteriologico de S. Paulo. Brasil Medico. **31:** 99–102.

———1922 Contribuições para o conhecimento da fauna helimntolojica brasileira—XVI. *Cruzia tentaculata* (Rud. 1819). Mem. Inst. Oswaldo Cruz **14:** 88–94.

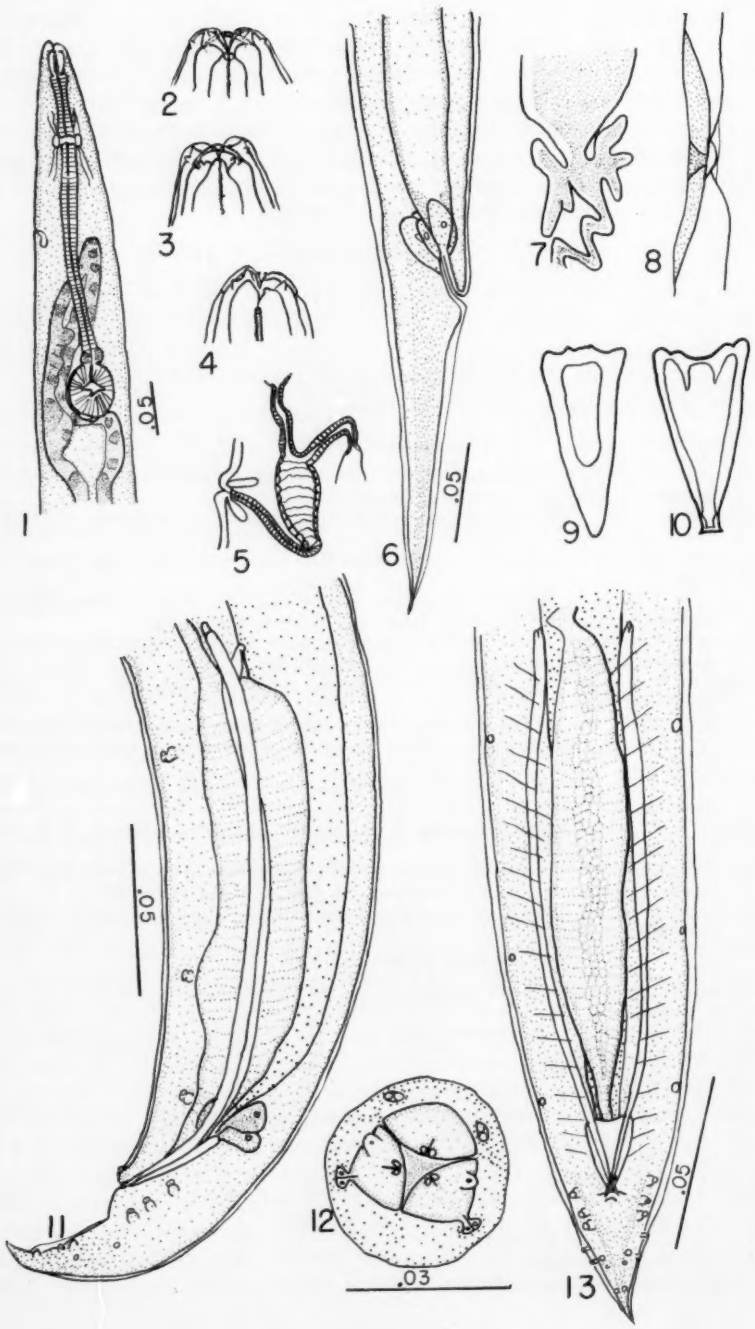
WOLFGANG, R. W. 1951 Studies on the endoparasitic fauna of Trinidad mammals. Canad. J. Zool. **29:** 352–373.

EXPLANATION OF PLATE

All figures except figure 9 were drawn with the aid of a microprojector and are of *Cruzia americana* unless stated otherwise.

FIG. 1. Anterior end, ventral view.
FIG. 2. Lips, ventral view.
FIG. 3. Lips, dorsal view.
FIG. 4. Lips, lateral view.
FIG. 5. Vulva, vagina, ovijector, vaginae uterinae.
FIG. 6. Tail of female, ventral view.
FIG. 7. Seminal glands.
FIG. 8. Cervical papilla.
FIG. 9. Gubernaculum, of *Cruzia tentaculata* after Ruiz (1947).
FIG. 10. Gubernaculum of *Cruzia americana,* dissection.
FIG. 11. Tail of male, lateral view.
FIG. 12. Lips, *en face* view.
FIG. 13. Tail of male, ventral view.

PLATE I



ECTOPARASITES FROM SWALLOW NESTS, WITH THE DESCRIPTION OF A NEW CERATOPHYLLID FLEA

RICHARD B. EADS

Bureau of Laboratories
Texas State Department of Health, Austin

The numerous limestone caves of Central Texas provide retreats for millions of Mexican free-tailed bats, *Tadarida mexicana*, and lesser numbers of several other species. These caves have been rather extensively investigated by personnel of this Department during studies designed to evaluate the importance of these insectivorous mammals as reservoirs and transmitting agents of rabies virus.

One of the largest and most interesting Texas bat harborage is Frio Cave located in Uvalde County. The first chamber, a room of considerable size, has several entrances including two openings in the roof which admit sufficient daylight to create an environment suitable for swallow nesting. The bats hang farther back in the darkened areas of the cave.

On March 30, 1955, several of the old swallow nests located in recesses along the walls and ceilings were examined for ectoparasites in association with my colleagues George C. Menzies and John S. Wiseman. The nests were composed of mud and lined with such materials as grass, wool and feathers. A good series of *Ceratophyllus* fleas (50), numerous *Haemolaelaps* mites and several *Ornithodoros* ticks were taken.

Specimens of the birds were netted and the species involved was determined to be *Petrochelidon fulva pallida*, or Coahuila cliff swallow. The ticks were indentified by Glen M. Kohls, Rocky Mountain Laboratory, Hamilton, Montana, as *O. concanensis* and R. W. Strandtmann, Texas Technological College, Lubbock, Texas, gave us the opinion that the mites are *H. megaventralis*.

Additional nests were examined in May, 1955 at which time the eggs had been laid and a few young birds were hatched. A good series of the *Ceratophyllus* flea was again taken. *H. megaventralis* mites were swarming all over the nests and *O. concanensis* ticks were common.

The same *Ceratophyllus* flea was also found in abundance in nests of the Coahuila cliff swallow in caves in Kerr County during June, 1955. A large number was taken from caves on the Wison and Johnson Ranches in this County. This represents the second species of *Ceratophyllus* to be taken in Texas. J. S. Wiseman collected a good series of *C. celsus* from *Petrochelidon albifrons* nests at Camp Bullis, Bexar County, Texas, June 14, 1955. The discovery of these two bird fleas raises the total number of known species of these ectoparasites in Texas to forty-six species and thirty-two genera (Eads, 1950).

We were not able specifically to place the flea, either from the literature or our collection and specimens were sent to George P. Holland, Department of Agriculture, Ottawa, Canada, a specialist on the *Ceratophyllus*. Mr. Holland agreed that the species was new and has been helpful with taxonomic assistance. A description, with figures, is presented in this paper.

Received for publication, September 13, 1955.

Ceratophyllus coahuilensis, n. sp.

This flea is distinctive among the known *Ceratophyllus* in the wide separation between the proximal and distal lobes of the male IX sternum. Superficially, the species resembles *C. niger* Fox in general shape of the movable and immovable processes of the clasper. In *niger* the apical membrane of the male VIII sternum is widest at its base, while in *coahuilensis* the membrane widens sharply toward its apex. The male modified abdominal segments differ markedly in shape and chaetotaxy from *C. gilvus* Jordan and Rothschild, also described from Mexican swallows. The female is less distinctive, but it is believed that the single row of five or six short, stout bristles running dorsoventrally on the inner surface of tergum VIII will be of value in separating this species from others of the *niger* group.

Male: Evenly rounded frontoclypeal margin of head with a distinct tubercle. Preantennal region of head with a lower, or ocular, row of three large bristles and an upper row of five or six smaller ones; about 15 small setae interspersed among the two rows of bristles and along the anterior margin of the antennal fossa. Eye conspicuous, subovate, well pigmented. Five-segmented labial palpi extending about to middle of fore trochanter. Genal process well sclerotized, subacute. Maxillary palpi extending almost to apex of fore coxae; scape of antenna with numerous marginal setae; second antennal segment with apical bristles, longest of which reach to penultimate division of club. Postantennal region of head with caudomarginal row of about six bristles, the most ventrad of which is twice the length of the others; sixteen–eighteen fine setae delimiting the margin of the antennal fossa, plus a long bristle and three smaller ones along upper third; around 15 fine setae along top of head.

Pronotum with comb of 14–15 heavy spines per side plus a row of about six bristles; most of the spines are about equal in length to the pronotal width. Mesonotum with a cephalic row of about ten small bristles, seven or eight scattered bristles, a mesial row of five large bristles alternating with small ones and a caudal row of five or six pseudosetae. Mesepisternum with five–seven bristles, the ventral one usually being over twice the length of the others; mesepimeron typically with four fairly long bristles, with three in a row on caudal margin. Metanotum with a row of five long bristles alternating with a similar number of smaller ones, preceded by a row of about four smaller bristles. Pleural ridge of metathorax strongly developed, terminating in an arrowhead-shaped expansion enclosed in a well-developed pleural arch.

Legs well developed as expected since this flea is active for a nest inhabiting species, jumping freely. Fore femur with about seven lateral median bristles in addition to several marginal ones; hind tibia with six pair of heavy, subequal bristles along posterior margin; fifth segment of all tarsi with five pair of lateral bristles in line. Unmodified abdominal terga typically with a row of three–five short bristles followed by a row of seven or eight longer ones; terga I to IV usually with two apical spinelets.

One long antepygidial bristle per side flanked by two or three small setae. Tergum VIII large, enclosing most of the modified abdominal segments as is typical of this subfamily, about seven long bristles along dorsal margin and two large lateral bristles, caudal margin with a broad, shallow sinus. Membranous structure with long spicules covering much of distal arm of sternum IX and aedeagus. Sternum VIII long and rod-like with four or five small bristles toward distal end; three long bristles and a membranous appendage toward apex; this appendage is narrow at base and widens strongly beyond middle; the distal half bears spicules with exception of cephalic margin. Sternum IX with proximal arm about the same length as the distal arm; distal arm with apical two-thirds produced in two downward projecting lobes which are more widely separated than is the case with other known *Ceratophyllus* and an apical flap with bluntly rounded caudal margin; the lobes are provided with seven or eight stout bristles of moderate length and several more slender bristles; apical flap with numerous scattered thin bristles of varying length.

Immovable process of clasper broad, about two-thirds the length of movable finger, broadly rounded at apex, caudal margin slightly sinuate, with three thin bristles at apex; acetabular bristles in usual position on ventrocaudal border, of approximately same length. Movable finger about four times as long as basal width, widest where anterior margin is produced into an acuminate, sclerotized process opposite apex of immovable process; cephalic margin with seven or eight short setae; caudal margin with five bristles and two or three short, dorsal setae as illustrated.

The aedeagal apodeme long and slender, about eight times as long as broad, measuring from middle of the constricted neck; apical appendage almost equaling length of aedeagal apodeme; proximal spur well developed. Penis rods and apodemal rod of sternum IX long and coiled. Aedeagal crochets shaped as the head of a long-nosed fish such as the dolphin.

Female: Chaetotaxy of head essentially as in male. Pronotal comb of fifteen–sixteen spines per side. Abdominal terga I to III, with two apical spinelets per side, one on IV. Three antepygidial bristles, the middle if which is five times the length of the outer pair. Anal

stylet about two and a half times as long as its basal width, with a long apical and two short subapical bristles. Eighth tergum with about twenty-two long, ventrolateral bristles, plus a row of three–five (usually four) short, heavy bristles on innersurface near caudal margin. Seventh sternum showing some variation in the caudal margin sinus depth; typically, the sinus is shallow, with the dorsocaudal margin formed into an evenly rounded lobe and the ventrocaudal margin fairly straight and not produced into a definite lobe. Seventh sternum with eighteen–twenty bristles arranged in three or four irregular oblique rows. Spermatheca with body long and narrow, about five times as long as wide; tail about two-thirds as wide and two-thirds as long as body.

Holotype male, allotype female and numerous paratypes of both sexes collected from nests of the Coahuila cliff swallow *Petrochelidon fulva pallida*, March 30, 1955, Uvalde County, Texas; holotype and allotype, No. 62924, deposited in collection of the U. S. National Museum.

SUMMARY

This description of a new flea of the genus *Ceratophyllus* from nests of the Coahuila cliff swallow increases the total number of these ectoparasites taken in Texas to 46 species referable to 32 genera.

LITERATURE CITED

EADS, R. B. 1950 Fleas of Texas. State Dept. of Hlth. Bull., Austin. 85 pp.

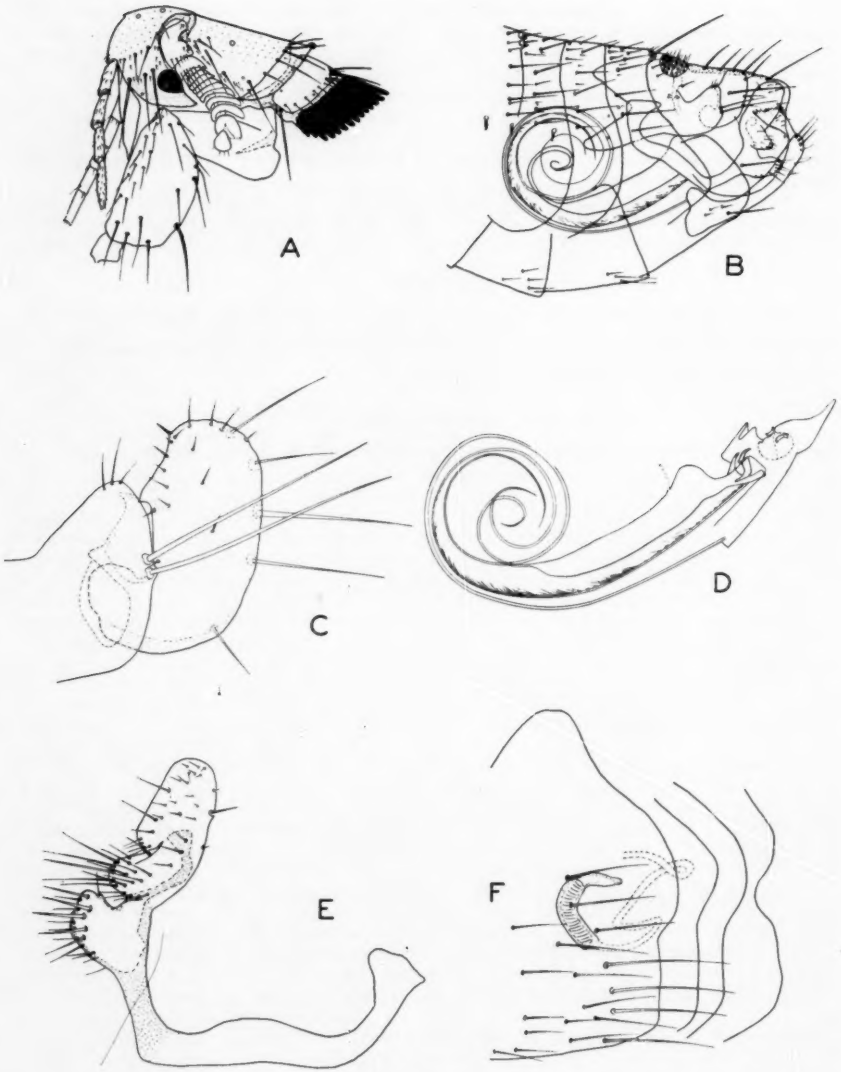


PLATE I

FIGURES

Ceratophyllus coahuilensis. A. Anterior end, male; B. Modified abdominal segments, male; C. Process and movable finger; D. Aedeagus; E. Ninth sternum, male; F. Female seventh sternum, showing variations.

IDENTIFICATION OF BETA ZnS IN THE INTESTINAL CELLS OF *STRONGYLUS* SPP.*

DAVID T. CLARK

Department of Microbiology and Public Health,
Michigan State University, East Lansing, Michigan

Zinc was first identified in nematodes by Rogers (1940) who quantitatively determined this metal in *Strongylus vulgaris* (Looss, 1900) Railliet and Henry, 1909, and *S. edentatus* (Looss, 1900) Railliet and Henry, 1909, by the use of the spectroscope. Because of the method used Rogers was unable to state with certainty, but suggested, that the zinc was combined with sulfur as ZnS in these two species of *Strongylus*. The zinc was believed by Rogers to be localized in the intestinal cell inclusions, called sphaerocrystals. This is not in agreement with conclusions of previous workers.

Quack (1913) identified the sphaerocrystals in *S. equinus* Mueller, 1780, as $CaSO_4$; and this was supported by Chitwood and Chitwood (1938). The Chitwoods found ferric iron associated with the granules. Because of the occurrence of these inclusions in *Strongylus* sp. they were named strongylin by the Chitwoods.

It is the purpose of this paper to report the results of a study on the sphaerocrystals in the intestinal cells of the three species of *Strongylus* indicated above to clarify the existing discrepancy as to their composition. It is considered important to know the composition of these intestinal cell inclusions to gain insight to possible metabolic pathways of these nematodes.

MATERIALS AND METHODS

Specimens of *Strongylus vulgaris, S. edentatus* and *S. equinus* were removed from ceca of horses killed at an abattoir and placed in 0.85% saline. The nematodes were then separated according to species and thoroughly washed in saline. Excess saline was poured off, and the living worms were frozen and stored in a deep freeze.

The inclusions were studied with the conventional microscope using transmitted, indirect and polarized light. Polarized light was produced by using two pieces of "Polaroid film," one between the light source and the substage condensor and the other between the objectives and oculars. For these observations the intestine was dissected from the nematode and mounted in 0.85% saline or 2N NaOH.

Preparation of Samples for X-ray Powder Diffraction:

Samples from which x-ray diffraction patterns were to be obtained were prepared in several manners. In the case of *Strongylus vulgaris* the previously frozen nematodes were thawed. The anterior and posterior tips were cut off and the body wall was slit longitudinally allowing the intestine to be removed inact. Intestines removed in this manner were slit longitudinally with small scissors and the contents of the intestine removed by washing in distilled water. Washed intestines

Received for publication, November 3, 1955.

* Part of a thesis submitted to the Graduate School, University of Illinois, in partial fulfillment of the requirements for the Ph.D. degree. This work was done under the direction of L. J. Thomas.

were pooled and divided into two portions. One portion was mixed with 1 ml. of 2*N* NaOH in a 15-ml. centrifuge tube and warmed in a boiling water bath for 15 min., centrifuged, and the supernatant removed. The residue consisting of isolated cell inclusions was washed several times with distilled water, collected and dried in a watch glass. The other portion of washed intestines was homogenized in 2 ml. distilled water in an all glass homogenizer, concentrated by centrifugation, collected and dried in a watch glass. Whole *S. vulgaris* were also homogenized in water, collected and dried in a watch glass.

It was found that the x-ray diffraction patterns obtained from the three samples prepared as described above were similar; therefore, the diffraction patterns of the inclusions in *S. edentatus* and *S. equinus* were obtained from whole washed intestinal cells only.

X-ray Powder Diffraction Procedures:

The x-ray diffraction technique was that of Debye-Scherrer (Klug and Alexander, 1954) using North American Philips X-ray diffraction equipment. Nickel-filtered copper radiation (wavelength 1.54 A) at 35 Kv and 18 ma, and a cylindrical camera 114.6 mm. in diameter with Eastman Kodak No-screen Double Emulsion X-ray Film were used. Dry samples prepared as described above were ground with a glass rod in a watch glass and loaded into glass capillaries made especially for x-ray diffraction procedures, (Obtained from Caine Sales Co., Chicago, Illinois). These capillaries have an outer diameter of 0.7 mm. and a wall thickness of 0.01 mm. One end of the capillary was flame-sealed. After loading, the open end was sealed with vaseline. The loaded capillary was centered in the camera and rotated by a motor for an exposure time of 6 to 18 hours depending upon the sample. Exposure time was determined by trial and error. Usual photographic procedures were used for film development. The 2*S* arc separations were measured with a Norelco Illuminator with a millimeter scale and a vernier reading to 0.1 mm. Relative arc intensities were estimated by eye with the aid of a multiple film calibration set (Robertson, 1943).

With the camera diameter used the 2*S* separations equal 4θ. The crystal lattice d spacings in Angstrom units corresponding to the 2*S* arc separations in millimeters were obtained from the tables of the National Bureau of Standards (1950) in which θ vs. d. Identification of the intestinal cell inclusions as beta ZnS was first accomplished by comparison of d spacings obtained from the samples with d spacings listed for known substances in the American Society of Testing Materials Index (1950 edition). The identification was then confirmed by comparison of the x-ray diffraction patterns of the inclusions with a pattern obtained from known beta ZnS (sphalerite).

RESULTS

Microscopically the strongylin intestinal cell inclusions in the three species of *Strongylus* appeared to be identical. They were of a rather constant size measuring approximately 5 microns in diameter. In transmitted light the granules were black, but in indirect light the granules were red-brown to white. They were non-birefringent, but they were surrounded by a shell which was birefringent with a cross of polarization. Although the granules were present in all the intestinal cells, they were more numerous in the cells of the anterior half of the intestine.

The granules were situated in a zone immediately below the bacillary border of the intestinal cells.

After treatment of the intestinal cells with 2N NaOH only the non-birefringent portion of the granule remained.

Table 1 presents a summary of the x-ray diffraction data obtained from the

TABLE 1.—Summary *of d spacings obtained from strongylin and beta ZnS*

Sample	*d* Spacing (Angstroms)	Relative Intensity	Sample	*d* Spacing (Angstroms)	Relative Intensity
Beta ZnS (ASTM Index)	3.12	100	Intestine of *S. vulgaris*	3.11	100
	2.69	5		1.90	75
	1.91	75		1.62	70
	1.63	50		1.23	5
	1.35	5		1.10	5
	1.24	18		1.04	5
	1.10	15		.95	1
	1.04	20		.91	3
	.96	20			
	.91	10	*S. vulgaris* (whole)	3.11	100
Beta ZnS (Sphalerite)	3.12	100		1.90	80
	2.67	5		1.62	65
	1.90	80		1.24	5
	1.62	60		1.10	5
	1.35	20		1.04	3
	1.23	20		.91	1
	1.10	30	Intestine of *S. edentatus*	3.12	100
	1.04	20		1.92	75
	.96	20		1.63	70
	.91	10		1.24	10
				1.10	5
Isolated strongylin granules from *Strongylus vulgaris*	3.12	100	Intestine of *S. equinus*	3.11	100
	2.70	1		2.70	1
	1.94	75		1.90	75
	1.63	60		1.61	70
	1.34	1		1.34	5
	1.24	10		1.24	20
	1.10	3		1.10	30
	1.04	3		1.04	25
				.95	2

samples of *Strongylus* spp. and sphalerite.

DISCUSSION AND CONCLUSIONS

The results of this investigation as to the color, size and location of the strongylin intestinal cell inclusions are in agreement with previous investigations. The birefringence of the granules has been found to be birefringence of the shell or hull surrounding the inclusion; not the whole inclusion as described by Quack (1913) and Chitwood and Chitwood (1938). The inclusion itself lacks birefringence.

It may be seen in Table 1 that the crystal lattice *d* spacings and relative arc intensities obtained from the samples from the three species of *Strongylus* are very close to the *d* spacings of known beta ZnS and are in agreement with the Testing Materials Index (1950).

Thus from the results of x-ray powder diffraction investigations of the intestinal cell inclusions referred to as strongylin, it may be conclusively stated that strongylin is beta zinc sulfide in *Strongylus vulgaris, S. edentatus* and *S. equinus.* This is in agreement with the results of Rogers (1940) who suggested that the granules were ZnS on the basis of quantitative determinations of zinc and sulfur in *S. vulgaris* and *S. edentatus.* The technique used by Rogers, in which the granules were dissolved in HCl, made it impossible to determine the chemical combination of the zinc and sulfur which was accomplished in the present study by x-ray diffraction procedures. That strongylin is ZnS in the living nematode is supported by the

series of patterns obtained from *S. vulgaris*. The patterns from whole worms and washed intestines which received no harsh treatment other than freezing, and the pattern from granules isolated by treatment with NaOH all matched the pattern of known beta ZnS.

Identification of strongylin in *Strongylus equinus* as ZnS is contrary to the findings of both Quack (1913) and Chitwood and Chitwood (1938) who believed the granules to be composed of $CaSO_4$. This discrepancy is believed to be due to the techniques used by these earlier workers. It is interesting that Chitwood and Chitwood (1938) found iron to be present in the residue. This is expected with the identification of beta ZnS since it is known that naturally occuring beta ZnS (sphalerite) contains as much as 20% iron (Dana, 1946).

The occurrence of zinc sulfide in the intestinal cells of *Strongylus* is probably incidental to the metabolism of the nematodes. By the process of digestion, zinc would be released from its association with the enzyme carbonic anhydrase of the blood ingested by the nematode. At the pH of the intestine and ingested blood zinc would be precipitated by any free sulfur ions which might be present in the intestine of the worm. Free sulfur ions could arise from H_2S produced by bacteria in the cecum and large intestine or by the nematode's own metabolism. Experimental evidence is lacking, but the above is suggestive that *Strongylus* has a desulfhydrogenase which produces H_2S from sulfur-containing amino acids of the ingested blood. The H_2S would ionize, and the S ions precipitate out Zn ions as ZnS.

The iron which occurs with the strongylin granules is probably also a sulfide which is precipitated along with the zinc.

If zinc or sulfur is toxic to *Strongylus*, the formation of insoluble ZnS would serve as a method of detoxification.

SUMMARY

The inclusions found in the intestinal cells of *Strongylus vulgaris, S. edentatus* and *S. equinus* called strongylin have been identified as beta ZnS by x-ray powder diffraction procedures.

Literature Cited

American Society of Testing Materials 1950 X-ray diffraction data card index. Philadelphia.

Chitwood, B. G. and Chitwood, M. B. 1938 Further notes on intestinal cell inclusions in nemas. Proc. Helminth. Soc. Wash. **5:** 16–18.

Dana, J. D. 1946 The system of mineralogy. 17th ed., 834 pp. Wiley and Sons, Inc., New York.

Klug, H. P. and Alexander, L. E. 1954 X-ray diffraction procedures for polycrystalline and amorphous materials. 716 pp. Wiley and Sons, Inc., New York.

National Bureau of Standards 1950 Table #10 U. S. Gov. Print. Office, Washington, D. C.

Quack, Maria 1913 Über den feineren Bau der Mitteldarmzellen einiger Nematoden. Arch. f. Zellforsch. **11:** 1–50.

Robertson, J. M. 1943 Technique of intensity measurements in x-ray crystal analysis by photographic methods. J. Sci. Instruments **20:** 175–179.

Rogers, W. P. 1940 The occurrence of zinc and other metals in the intestines of *Strongylus* spp. J. Helminth. **18:** 103–116.

THE LOCATION OF THE SCHISTOSOME VECTORS, *AUSTRALORBIS GLABRATUS* AND *TROPICORBIS CENTIMETRALIS*, ON AND IN THE SOIL ON DRY NATURAL HABITATS

LOUIS OLIVIER

U. S. Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health
National Microbiological Institute[1]
Bethesda, Maryland

Northeastern Brazil, where schistosomiasis mansoni is endemic, has a marked seasonal rainfall cycle, and as a consequence, many of the habitats of *Australorbis glabratus* and *Tropicorbis centimetralis,* the snail vectors of the disease in that region, contain water about 5 to 7 months of the year and are without standing water throughout the remainder of the year.

It has been shown (Olivier and Barbosa, 1955a, b) that the snail vectors of schistosomiasis in that region may survive the dry season in the protection of vegetation and debris at the ground level. From these field studies, it was concluded that the snails apparently do not enter the soil to avoid the consequences of drying.

Laboratory studies have supported this viewpoint (Olivier, 1956; Olivier and Barbosa, 1956). Snails were put on the surface of mud, or in shallow water over mud, and then allowed to dry gradually. Almost all of the snails stayed at, or just under, the soil surface although a few penetrated more than one centimeter into the soil. From this evidence it was concluded that the snails probably do not penetrate deeply into the soil in significant numbers and that entry into the soil is not necessarily related to its drying.

It was also found that snails buried in relatively dry soil lived about as long as those at the soil surface, but that snails buried in water-saturated mud soon died (Olivier and Barbosa, 1956; Perlowagora-Szumlewicz and Oliveira Dias, in press).

In an effort to learn more about the way the snails pass the dry season, and to study further the extent to which the snails tend to enter the soil, a series of collections were made in dried habitats. The data were obtained from meter-square plots marked off in depressions normally flooded during the wet season and known to be habitats for the snails. Although there were minor variations in procedure, the collections were made essentially as follows: All shells visible in the quadrat, before disturbing the vegetation or debris, were removed first. This was called the first collection from the surface. The vegetation and debris were then removed with minimum disturbance of the soil, and all snails revealed by this procedure were collected. This was considered the second collection from the surface. In some cases these two collections were combined. Then, the top centimeter (approximately) of the soil was carefully removed and passed through a fine screen which

Received for publication, October 26, 1955.

This report is based on studies conducted at the Instituto Aggeu Magalhães by the Laboratory of Tropical Diseases, National Institutes of Health, U. S. Public Health Service, under the sponsorship of the Pan American Sanitary Bureau in cooperation with the Divisão de Organização Sanitaria and the Serviço Nacional de Malária do Departamento Nacional de Saúde de Brasil.

[1] Laboratory of Tropical Diseases.

TABLE I.—*Distribution of snails in 15 collections made in dry habitats in Pernambuco, Brazil**

Collection no.	Locality	Date	Number of empty shells	Empty shells						Number of living snails	Living snails					
				Surface		From soil					Surface		From soil			
				1	2	Top cm	2nd cm	3rd cm	Next 3 cm		1	2	Top cm	2nd cm	3rd cm	Next 3 cm
1	Paulista	8 Oct. 53	148	38	95		15		–	64	8	47		9		–
2	Paulista	4 Nov. 53	112	25	35		33		19	56	12	21		23		0
3	Paulista	28 Dec. 53	277	98	123	33	18	5	–	53	21	23	6	3	0	–
4	Paulista	10 Feb. 54	398	100	128	99	36	27	8	69	2	34	24	3	6	0
5	Paulista	10 Mar. 54	659	188	173	157	67	74	–	150	10	49	78	13	0	–
6	Paulista	31 Mar. 54	738	383	48	213	91	–	3	60	12	17	22	9	–	0
7	Arruda	7 Oct. 53	40	13	5	15	7	–	–	11	1	6	4	0	–	–
8	Olinda 1	9 Oct. 53	58	8	5	27	18	–	–	46	34	7	5	0	–	–
9	Olinda 2	9 Jan. 54	80	11	19	13	4	33	–	14	4	0	4	5	1	–
10	Beberibe	26 Feb. 54	44	5	10	4	13	–	12	34	20	11	3	0	–	0
11	Recife	10 Nov. 53	223	83		113	27	–	–	53	12		37	4	–	–
12	Recife	9 Feb. 54	445	57		229	128	–	31	4	3		1	0	–	0
13	Agua Fria	18 Sep. 53	46	29		7		10		52	41		5		6	
14	Vitória	18 Dec. 53	862	34		461	148	113	106	142	94		45	3	0	0
15	Vitória	8 Feb. 54	818	245		297	194	70	12	18	13		4	1	0	0

* Collections 1–13; *Australorbis glabratus*. Collections 14 and 15; *Tropicorbis centimetralis*.

collected all snails having a diameter of more than one millimeter. Following this, the second, and sometimes the third, centimeter were removed and examined in the same way. Finally, a block of soil one-half meter square and 3 centimeters deep was removed from the center of the quadrat and examined for snails.

The records of living snails found in 15 quadrats so searched are shown in Table I.

The first 6 collections were made in adjacent quadrats in a large field in Paulista, Pernambuco described in Olivier and Barbosa (1955a) as Field 4. Standing water disappeared from this field during September, 1953 and did not return until April, 1954. When the collections were made the vegetation in the quadrats consisted of tall, rank, grass which effectively shaded the soil. The latter consisted of a layer of dark loam and debris about 2 cm thick overlying somewhat heavier sandy loam.

In these quadrats living snails were found only once at a depth greater than 2 cm, and in this case there was reason to believe that the sample had been contaminated with soil from the surface. In total, 2,784 shells were found. Of these, 61 percent were at the surface or in the top centimeter of the soil. In the last 4 collections in this series 18 percent of all the shells, but only 10 percent of the living snails, were found below the first centimeter of the soil. The ratio of living snails to empty shells changed gradually from about 1 in 3 on October 8, 1953 to 1 in 13 on March 31, 1954 indicating that the snails died off gradually as the dry season progressed. The death rate among the snails buried in the soil was not significantly different from that of the snails at the soil surface.

Collection 7 was taken from a shady depression in a vacant lot where the soil was deeply covered with debris and humus. The soil was moist, soft, and sandy. Most of the shells, and all the living snails, were found at or near the soil surface.

Collection 8 was taken from a shallow drainage ditch along a principal highway. The ditch contains water only about four months of the year, but during that time it harbors many snails. Dense grass grows in the ditch throughout the year, and even in the dry season the soft porous soil is moist. The collection was made about two months after the ditch dried up. Most of the living snails were at the soil surface and none was found below the first centimeter.

Collection 9 was taken from the bottom of a small, shady depression which is flooded during the rainy season and harbors many snails. In the dry season the soil is always moist, soft, and porous, but there is no standing water. Living snails were found on and in the soil, but, only 1 of 34 shells from the deepest soil sample harbored a living snail.

Collection 10 came from a small borrow-pit in an open field where the water table was relatively high even in the dry season. The sampled area consisted of soft mud with little vegetation or debris. Shells were found at all levels in the soil, but all the living snails were at or near the surface.

Collections 11 and 12 were taken from adjacent quadrats in a grassy field flooded only about 4 months of the year. At the time of the first collection the soil was somewhat moist, and there was a dense growth of grass. When the second collection was made the soil was dry but soft and loose, and the vegetation had withered. Shells were numerous at the soil surface and in the first 1 or 2 centimeters. All but a few of the living snails were at or near the soil surface.

Collection 13 was made in a field still moist from the rainy season floods. There

was no vegetation on the sampled plot, and the soil was very soft and contained much organic matter. Many living snails were at the soil surface, and though few shells were found in the soil, some harbored living snails.

The last two collections shown in the table were taken from a depression adjacent to the river in Vitória de Santo Antão. For about four months, during the rainy season, this depression contains water to a maximum depth of about 10 cm. The sampled areas, which lay in the full sun, had little vegetation, and the soil was soft and very sandy.

At the time of the first collection the depression had no water and the soil was dry at the surface but moist below the surface. Shells were numerous at all sampled levels of the soil, but the living snails were all found at or near the surface. When the second collection was made many shells were found, but there were very few living snails, and all of these were relatively close to the soil surface.

The distribution of the living snails differed from that of the empty shells. Examination of collections 3 to 13 shows that 38 percent of the empty shells were at the soil surface and 36 percent were in the top centimeter of the soil. On the contrary, 59 percent of the living snails were at the soil surface and 34 percent were in the top centimeter of the soil. Living snails were much more scarce in the deeper soil samples than were the empty shells. Only one percent of the living snails were below the second centimeter (12 collections) while 11 percent of the empty shells were below that depth. Sixteen percent of all shells at the surface or in the top centimeter of the soil were alive while only 4 percent of all the shells below the first centimeter were alive.

DISCUSSION

In the collections just described, a large majority of all the living snails were at the soil surface or in the top centimeter of the soil. Those at the surface were left stranded there when the water level fell and the pools dried up. No doubt, some of the snails in the top centimeter of soil had entered the soil as a result of their own activity. However, this may have occurred in the course of normal movement before the water disappeared and so was not necessarily related to the fall of the water level. Others in the top centimeter of the soil may have been buried there accidentally.

Some living snails were found in the second centimeter of soil and a few deeper than that. Again, some of these might have penetrated to the depth at which they were found. Evidence that such penetration may occur occasionally was obtained in laboratory experiments (Olivier and Barbosa, 1956). However, soil penetration is believed to be atypical and unusual for several reasons. First, in previous laboratory experiments (Olivier and Barbosa, 1956) few snails penetrated more than a short distance into the soil. Second, it seems unlikely that penetration can be possible unless the mud is very soft, since these snails have a large shell and a relatively small, weak foot. Moreover, water-saturated mud may be a very unsuitable environment for the snails.

On the other hand, the snails can survive long periods in soil that is not water-saturated (Olivier and Barbosa, 1956). Therefore, it is believed that most of the living snails found deep in the soil probably did not reach that level when the mud was very wet since they would probably have died under such conditions. However, they may have been buried there accidentally as the soil on the bottoms of the pools

dried up. They could have been pressed into the soft soil under the feet of men and animals or they could have been covered by other disturbances of the surface soil. Of course, empty shells could also be buried in this way.

All this tends to confirm the impression, based on evidence from other field and laboratory experience to the effect that the snail vectors of *Schistosoma mansoni* in Pernambuco tend to remain at the ground surface when their habitats dry and do not enter the soil in order to avoid the consequences of drying.

It should be borne in mind, in this connection, that recovery of a snail from the soil is not evidence that it penetrated into the soil. Moreover, snails embedded in the soil may not be able to emerge unaided.

The vertical distribution of empty shells in the collections was not the same as that of the living snails. A much larger proportion of the latter were at the soil surface or in the first centimeter of soil. Although many factors might have influenced the distribution of both groups, these results tend to support the view that the snails may survive better at the soil surface than deep in the soil.

Although the evidence presented tends to show that the snails do not enter the soil to avoid the consequences of drying, and that the small number of snails found deep in the soil may have reached that location by accident in most cases, it is not possible to conclude that *A. glabratus* and *T. centimetralis* behave the same way throughout their range. That can be determined only by careful study in a variety of habitats.

Literature Cited

Olivier, Louis 1956 Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. I. J. Parasit. (in press).

Olivier, Louis and Barbosa, F. S. 1955a Seasonal studies on *Australorbis glabratus* Say from two localities in Eastern Pernambuco, Brazil. Pubs. Avulsas Instituto Aggeu Magalhães (in press).

——— and ———1955b Seasonal studies on *Tropicorbis centimetralis* in Northeastern Brazil. Pubs. Avulsas Instituto Aggeu Magalhães (in press).

——— and ———1956 Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. II. J. Parasit. (in press).

Perlowagora-Szumlewicz, Alina and Oliveira Dias, Geraldo de Experiências de laboratório sôbre a possibilidade os planorbídeos viverem dentro da lama nela se enterrarem na ocasião do tratamento com planorbicidas. Proc. XII Congresso Brasileiro de Higiene (in press).

THREE NEW CERCARIAE FROM *AMNICOLA PILSBRYI* WALKER, 1906, WITH SOME NOTES ON THEIR LIFE HISTORIES

FRANK J. ETGES
Department of Zoology, University of Cincinnati

Incidental to previous studies on the life histories of two microphallid trematodes, three other cercariae were found infecting the same species of snail, *Amnicola pilsbryi* Walker, 1906. They were identified as notocotylid, psilostome, and pleurolophocercous types respectively. Subsequent examination indicates that none of them has yet been described.

Lundahl (1955) shows that the amnicolid population of Douglas Lake, Michigan has a trematode infection rate of 1% in deeper waters, and 2% near the shore. The present study, made in Barrett's Pond, Putnam County, New York, indicates a much higher rate of infection, particularly in the months of September through December. Analysis of the July–August infection rate in *Amnicola pilsbryi* as revealed by dissection is: 3–6% with *Maritrema obstipum;* 3–4% with *Levinseniella amnicolae;* 2–3% with a notocotylid species; 1–2% with a psilostome species; and 0.1% with a pleurolophocercous species. No multiple infections were found in three years of study. Thus a minimum of 9+% of these snails is infected in mid-summer, with a gradual increase toward fall as previously shown for the two microphallid species (Etges, 1953). A condition which might explain this difference in rate of infection is the presence of many cold springs along the edge of Barrett's Pond, where *Amnicola* lives from March through December, and is presumably exposed more often to avian and mammalian fecal droppings. In Douglas Lake the snails spend the hot months in the deeper, cooler offshore waters, away from such sources of infection.

MATERIALS AND METHODS

Snails were collected and separated in groups of 25 in fingerbowls. Infected specimens were isolated as soon as possible, and cercariae were studied upon their emergence. Neutral red, nile blue sulfate, and brilliant cresyl blue were used as vital stains; the phase contrast microscope was also employed in examination of living material. Intramolluscan stages were obtained by dissection, studied alive and in permanent mounts. All specimens used for measurement were killed and preserved in cold Bouin's fixative, and stained with Semichon's acetic acid carmine. Cercariae studied after emergence are referred to below as mature specimens, in contrast to specimens obtained by dissection, which are classified as immature.

Drawings were made with the camera lucida and microprojector, as well as free-hand. Observations of fine details made on a number of living specimens were consolidated by adding them to camera lucida drawings. All dimensions are stated in millimeters.

Infection experiments were carried out using laboratory raised hosts, or uninfected animals from other localities than Barrett's Pond.

Received for publication, October 21, 1955.
The writer expresses his thanks to Dr. R. M. Cable for aid and advice in the preparation of this manuscript.

Cercaria amnicolensis n. sp. (Psilostomatidae)
(Figs. 1, 2, and 3.)

Specific diagnosis: gymnocephalous, distome, leptocercous, unpigmented cercariae of medium size. Dimensions of fixed, mature specimens: body, 0.130–0.160 long by 0.085–0.112 wide, ovoid to subspherical with a thick body wall; tail, 0.185–0.230 long by 0.028–0.042 wide at the base, with a slender central excretory duct leading to a caudal bladder in the proximal end of the tail; oral sucker, 0.030–0.039 in diameter, subcircular in outline, subterminal, usually slightly larger than the acetabulum; acetabulum, 0.025–0.034 in diameter, circular in outline, post-equatorial; prepharynx, short; pharynx, 0.007–0.015 long by 0.007–0.015 wide, subspherical to ovoid, usually truncated posteriorly; esophagus, up to 0.025 long, lacking diverticulae; cecae, long, slender, reaching almost to bladder; excretory vesicle, rounded or constricted into anterior and posterior chambers, with somewhat thickened walls, filled with small refractile granules 0.001–0.003 in diameter; primary excretory ducts, conspicuous, sinuous, filled with granules as in the bladder, extending forward to the level of the pharynx, then turning posteriad as a much smaller duct; flame cells small, in groups of two, eight on each side of the body; cystogenous glands, numerous, scattered, filled with granules which stain deeply with neutral red.

Rediae: 0.225–0.495 long by 0.090–0.142 wide, thin-walled, with slight constrictions, slightly motile, lacking locomotor appendages, containing 7 to 22 germ balls and immature cercariae; pharynx, 0.030–0.058 in diameter, subspherical; gut, slender, filled with coarse granules, extending posteriorly 1/3 to 1/2 body length; birth pore inconspicuous, just posterior to pharynx; situated in hepatopancreas and gonad tissue spaces of *Amnicola pilsbryi.*

Cercaria amnicolensis has been encountered only sporadically in *Amnicola pilsbryi,* with three infections noted in 1951, eight in 1952, and three in 1953. The chalk-white cercariae are shed in small numbers, and swim almost continuously, usually near the bottom of containers with brief interludes of crawling by means of their suckers. No periodicity of emergence or activity was noted. Infrequently cercariae underwent decaudation and formed thin-walled cysts on glass slides under some coverglass pressure, but encystment was never seen in open fingerbowls. At room temperature they live for 20 to 42 hours, with longevity extended under refrigeration. A maximum of nine cercariae emerged from a single snail in a 24 hour period.

Observation of living and fixed material failed to reveal the presence of cephalic glands or ducts (Figs. 1 and 2). Terminal excretory ducts were obscured by the opacity of the body wall, cystogenous glands, and parenchyma in mature cercariae. In immature specimens a maximum of eight flame cells was found in groups of two, but the excretory duct formula cannot be stated.

Attempts to infect a second intermediate host were all negative. Salamander adults and larvae, anuran larvae, guppies, various aquatic insect larvae, and isopods were used in these experiments.

Cercarial development is apparently completed outside the redia, since numerous immature individuals lie free in the tissue spaces, and fully developed cercariae were never seen inside rediae. Daughter rediae have not been identified among any of the rediae described here.

Cercaria amnicolensis is very similar to the cercaria of *Ribeiroia* (= *Psilostomum*) *ondatrae* (Price, 1931) as described by Beaver (1939), but may be distinguished from it by its lack of locomotor appendages in rediae, markedly smaller size, and difference in relative sucker size. *Cercaria ameeli* and *C. limosae* described by Hedrick (1943) both lack prominent excretory channels, possess much broader tails, and have a peculiar tendency to float when relaxed, all in direct contrast to *C. amnicolensis.* The cercaria of *Psilotrema spiculigerum* Mühling (= *Cercaria tuberculata* Fil.) differs from the present species in possessing prominent cuticular spines, prominent locomotor processes in the redia, and is considerably

greater in all its dimensions as described by Mathias (1925) and Wesenberg-Lund (1934). *Cercaria helvetica* XVII (= *Sphaeridiotrema globulus* Szidat, 1937) as described by Dubois (1929) has a tail almost as broad as its body, a redia with locomotor appendages, but is nearly the same size as *C. amnicolensis.*

Cercaria parapleurolophocercoides n. sp.
(Figs. 4, 5, 6, and 7.)

Specific diagnosis: small, biocellate cercariae of the Pleurolophocerca type. Dimensions of fixed, mature specimens: body, 0.085–0.172 long by 0.036–0.062 wide, ovoid to elongate, posterior end truncated; cuticular spines arranged in an anterior circle of 28 to 31 prominent spines, a second circle of smaller spines in equal number, and 12 to 18 rows of minute spines in quincunx, extending to the level of the pharynx; oral sucker a penetration organ, 0.017–0.032 in diameter, subspherical, highly protrusible; acetabulum, 0.010–0.021 in diameter, post-equatorial, variable in development from a slight elevation to a well differentiated sucker; tail, 0.280–0.346 long by 0.018–0.030 wide at the base, exclusive of fin-folds, sigmoid in shape; dorsoventral fin-fold continuous around end of tail, 0.002–0.012 high; lateral fin-folds extending less than ½ tail length from base of tail, with a notch in their edges at the position of caudal excretory duct pores (present in immature specimens only), 0.010 in maximum height; eyespots, 1/5 to 1/4 of body length from anterior end, 0.007–0.009 in diameter, ovoid with a shallow depression in the antero-dorsal face, composed of granular pigment; pharynx, 0.005–0.008 in diameter, poorly differentiated, just posterior to oral sucker; esophagus and cecae lacking; cephalic glands of two sizes located dorsally in the anterior ½ of body; seven large glands ca. 0.008–0.015 in diameter, and three small glands ca. 0.004–0.007 in diameter, on each side; cephalic gland ducts divide into two groups on each side, just behind the oral sucker, then pass forward to their orifices on the anterior edge of the oral sucker; a median group of four orifices and a lateral group of six results in a pattern of 6-4-4-6; two groups of 3 to 5 prominent carminophilic cells situated postero-lateral to oral sucker; genital anlage, a dendritic mass of deeply staining cells, just antero-dorsal to the acetabulum; excretory vesicle ovoid to round, slightly bicornuate, thick-walled, emptying through a short duct leading to a small pore in the ventral body wall (in mature specimens); caudal, bifurcate excretory duct degenerated prior to emergence with only remnants present in some mature individuals; eight flames cells on each side of body in positions shown in Fig. 4.

Rediae: 0.145–0.518 long by 0.065–0.090 wide, sinuous, frequently constricted, slightly motile, lacking locomotor processes, with 2 to 15 germ balls or immature cercariae; pharynx, 0.016–0.023 in diameter, subspherical; gut, a small mass of cells just behind the pharynx; birth pore inconspicuous, just back of mouth opening; in the hepatopancreas of *Amnicola pilsbryi.*

Collections of *Amnicola pilsbryi* made over a three year period had but a single infection of *Cercaria parapleurolophocercoides.* Whether this snail is an abnormal host, or if such a low rate of infection is characteristic is problematic. The definitive host must currently live in or visit the area, since *Amnicola* lives only 21 months in the laboratory, and probably not much more than a year in nature as pointed out by Lundahl (1941). *C. parapleurolophocercoides* emerges in large numbers (more than 50/24 hrs.) from the snail. The cercariae swim violently but sporadically, spending long periods lying on their sides on the bottom of containers. They show no marked sensitivity to light stimuli, but exhibit a response to induced water currents. Tapping the container is less effective in causing swimming activity. Cercariae usually emerge during the day, but emergence is not prevented by keeping the host in the dark continuously.

Attempts to infect a second intermediate host failed. In addition to the animals used in experiments with *C. amnicolensis* (vide supra), minnows, goldfish, and young sunfish were employed. Cercariae crawled about on the skin and scales, but did not penetrate. Subsequent examination of the exposed animals failed to reveal cysts in the skin, scales, fins, gills, body cavity, or internal organs.

The most distinctive characteristics of *C. parapleurolophocercoides* are: lateral

caudal fin-folds, two circlets of spines around the anterior end, and cephalic glands opening in a 6-4-4-6 pattern. By combination of these characteristics the present species may be distinguished from the cercaria of *Caecincola parvulus* as described by Lundahl (1941); *Cercaria sp.* from *Amnicola lustrica,* described by Hopkins (1938); *Cercaria sp.* from *Oncomelania hupensis* described by Kuntz (1952); *C. coronanda* described by Rothschild (1938); and *C. orospinosa* described by Ullman (1954). It is of interest to note that the last three cercariae were shown experimentally to develop into acanthostomid metacercariae in fish.

Martin and Kuntz (1955) described the cercaria of *Stictodora tridactyla,* which is similar to *C. parapleurolophocercoides* in bearing lateral fin-folds, but differs in respect to the number of cephalic glands and orifices, and length of dorsal fin-fold. Following his characterization of the Pleurolophocerca group, Sewell (1922) described two pleurolophocercariae, *C. indicae* VII and *C.I.* VIII with lateral fin-folds. Cable (1938) states that many species of pleurolophocercariae have an elevated caudal cuticula resembling lateral fin-folds; elevation of this cuticula is inconspicuous in the present species, and the lateral fin-folds show plicae like those in the dorsoventral fin, similar to the condition described by Martin and Kuntz in cercariae of *S. tridactyla.* Whether Sewell's two species had basal lateral fin-folds or a raised cuticula, his verbal description states that lateral fin-folds may be present in members of the Pleurolophocerca group. Accordingly, the present species as well as *S. tridactyla* should be included in that category.

Jones (1951) described *Cercaria setaecauda* from bithynoid snails (species unspecified) in Singapore. In many respects this cercaria resembles the pleurolophocercous type; i.e., body and tail form, eye spots, cephalic glands and ducts. The excretory system and cephalic gland duct orifice arrangement are undescribed. *C. setaecauda* is shown with 24 cephalic glands on each side, yet only 3 ducts run forward from them. The tail is described as lacking fin-folds, but bearing fine wavy setae at irregular intervals. A stylet is shown on the oral sucker along with several smaller spines. In view of these peculiarities, Jones failed to assign this species to any group of cercariae. It is suggested by the writer that the "setae" are the characteristic refractile crests of undulations in the fin-fold whose margins were not seen, that the "stylet" is a boring spine, and that *C. setaecauda* be classified as parapleurolophocercous.

Since pleurolophocercariae are known to be larvae of several trematode families, relationship of the present species is uncertain. The circlets of spines around the anterior end suggest a possible relation to the Acanthostomatidae; however it must be noted that these spines do not surround the mouth as in adult acanthostomids. Cable and Hunninen (1942) present convincing evidence of the probable identity of the Acanthostomatidae and Cryptogonimidae, but state that ". . . final disposition of the families Acanthostomatidae and Cryptogonimidae must await knowledge of acanthostomid life histories . . .". To the best of the writer's knowledge, final disposition of these families is still uncertain.

Cercaria wesenberg-lundi n. sp. (Notocotylidae)
(Figs. 8, 9, and 10.)

This cercaria is moderately pigmented, giving it a pink color in life. Cystogenous glands, present in large numbers, are most numerous on the dorsal side. Posterior

locomotor organs are minute but conspicuous in immature specimens; however they may not be visible in mature specimens, or permanent mounts. Cercarial emergence is slightly more frequent in early morning hours, but may occur at any time. No evidence of phototaxis was observed, vigorous swimming lasting from 10 to 45 minutes before encystation begins. Cysts form on glass, filter paper, lettuce, and snail shells. A maximum of 18 cysts has been recovered from an isolated snail after 24 to 36 hours. The tail continues vigorous movement during encystment, and may continue for more than 5 hours after decaudation. Under refrigeration (4–10° C.), cercariae encysted readily and tail movements were prolonged. Cercariae of this species have never been observed before August except by dissection. Intramolluscan development takes place in rediae, but is not completed until cercariae have escaped into the tissue spaces of the host. The rediae contain immature cercariae and germ balls, never mature cercariae or daughter rediae. Pigment in immature cercariae is concentrated around the eye spots, from which it spreads dendritically to all parts of the body, delineating digestive cecae, nerve cords, and excretory channels similar to the process described for *Cercaria ephemera* by Rothschild (1936).

Specific diagnosis: triocellate, gymnocephalous, monostome cercariae of the Ephemera type. Dimensions of fixed, mature specimens: body, 0.128–0.180 long by 0.054–0.109 wide, rounded to ovoid; tail, 0.188–0.340 long by 0.018–0.032 wide at the base, cylindrical, powerful, lacking spines or setae; oral sucker, 0.021–0.032 in largest diameter, terminal to subterminal, protrusible, usually wider than long; pharynx, 0.008-0.010 in diameter, poorly differentiated in some, situated between oral sucker and median eye spot; cecae, poorly developed, extending to level of genital primordium; posterior locomotor organs, inconspicuous, just lateral to base of tail on ventral side; excretory vesicle, rounded to ovoid, thin-walled, small to medium in size; primary excretory channels conspicuous, extending to level of eye spots, confluent medially just behind median eye spot, with a short median extension passing through median eye spot, containing few refractile granules; lateral eye spots, 0.012–0.017 in largest diameter, cup-shaped with open side dorso-laterally directed, composed of large pigment granules densely packed; median eye spot, 0.006–0.011 in largest diameter, a ring of pigment granules around the anterior diverticulum of the excretory system; genital primordium a bipartite mass of deeply staining cells, antero-dorsal to the excretory bladder between the excretory channels.

Rediae: 0.112–0.485 long by 0.082–0.165 wide, sac-like, rarely constricted, slightly motile, lacking locomotor ridges or appendages; birth pore inconspicuous, just posterior to pharynx; containing 1 to 9 germ balls or immature cercariae; pharynx, 0.032–0.043 in diameter, subspherical; gut, voluminous, extending ½ to ¾ body length, containing particles; situated in hepatopancreas and gonad tissue of *Amnicola pilsbryi*.

In an excellent review and analysis of the genus *Notocotylus* Diesing, 1839, Dubois (1951) recognizes two subgeneric types in anserine birds distinguished by their adult and larval morphology, and their molluscan hosts. He recommends the following synonymies: cercaria of *Notocotylus t. triserialis* Diesing, 1839 (= *N. intestinalis* Tubangui, 1932, *N. stagnicolae* Herber, 1942, *N. urbanensis* Cort, 1914, *Cercaria vaga* L. and U. Szidat, 1933, and *C. urbanensis* Cort, 1914); and cercaria of *Notocotylus imbricatus* Looss, 1893 (= *Cercaria helvetica* I Dubois, 1929, *C. imbricata* Looss, 1893, *N. lucknowensis* Lal, 1935, and *N. anatis* Ku, 1937). *Cercaria wesenberg-lundi* is most like the former species in respect to bearing a median eye spot, and in general body form, but differs in its markedly smaller size of both redia and cercaria, its poorly developed digestive system, length of redial gut, and in the type of molluscan host utilized. Similarly the present species may be differentiated from the following species in respect to its pigmentation, number of eyespots, smaller size of redia and cercaria, and small size of its posterior locomotor organs: cercariae of *Quinqueserialis quinqueserialis* as described by Herber

(1942), *Nudacotyle novicia* (= *Cercaria marilli*) as described by Ameel (1939), *Notocotylus attenuatus* as described by Rothschild (1936), and *N. imbricatus* as described by Wesenberg-Lund (1934). *Cercaria ephemera*, described by Rothschild (1936) and Wesenberg-Lund (1934), differs from the present species in having eye spots of subequal size, a more elongated redial pharynx and gut, and much larger size of both the cercaria and redia. On these grounds, the present species is recognized as distinct, and is named in honor of Carl Jürgen Wesenberg-Lund, now nearly 90 years old, whose contributions to the study of trematodes are well known.

Cysts of *C. wesenberg-lundi* are 0.120 to 0.140 mm. in diameter exclusive of any cement substance around them, flattened on the under-side and convex above. The cyst wall is thin and delicate when first formed, but later becomes thicker and brown in color. Cysts may be removed from their substrate with no damage to the enclosed worm. After 2 to 3 weeks of encystment, the metacercaria still closely resembles the cercarial body. Feeding 25 cysts to a number of chicks, mice, canaries, and pigeons resulted in recovery of a single developing worm in a chick intestine, four days after ingestion. These infection experiments were carried on concomitantly with studies on two microphallid species (Etges, 1953), in which four days is more than ample for the development of adult characteristics. Unfortunately this period is not long enough for this notocotylid species, which had grown to three times metacercarial size, but was still relatively undifferentiated. Some differences noted were: dispersal of eye spot pigment, increased thickness of excretory channel walls, separation of genital primordium into two parts, slight differentiation of uterus, and increased differentiation of the digestive system. The oral sucker showed a slight increase in size. Subsequent attempts to infect a variety of vertebrates have all failed, and identification of the immature worm is not possible with any degree of certainty.

SUMMARY

Intramolluscan and free-living stages of *Cercaria amnicolensis*, *C. parapleurolophocercoides*, and *C. wesenberg-lundi* n. spp. are described and differentiated from related species. *C. wesenberg-lundi* is shown to develop beyond the metacercarial stage in a chick, but specific identification of the adult is postponed until further work has been completed. Notes on infection experiments with *C. amnicolensis* and *C. parapleurolophocercoides* are reported, and observations on the natural incidence of all three species in *Amnicola pilsbryi* are made.

REFERENCES CITED

AMEEL, D. J. 1939 Cercariae infecting *Pomatiopsis lapidaria* Say. Amer. Mid. Nat. **21**: 651–656.

BEAVER, P. C. 1939 The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). J. Parasit. **25**: 383–393.

CABLE, R. M. 1938 Studies on larval trematodes from Kentucky with a summary of known related species. Amer. Mid. Nat. **19**: 440–464.

——— AND HUNNINEN, A. V. 1942 Studies on the life history of *Siphodera vinaledwardsii* (Linton) (Trematoda: Cryptogonimidae). J. Parasit. **28**: 407–422.

DUBOIS, G. 1929 Les cercaires de la région de Neuchatel. Bull. Soc. Neuchat. Sci. Nat. **53**: 1–177.

———1951 Étude des trematodes Nord-Americains de la collection E. L. Schiller et revision du genre *Notocotylus* Diesing, 1839. Bull. Soc. Neuchat. Sci. Nat. **74**: 41–76.

ETGES, F. J. 1953 Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella amnicolae* n. sp. (Trematoda: Microphallidae). J. Parasit. **39:** 643–662.

HEDRICK, L. R. 1943 Two large tailed cercariae (Psilostomidae) from northern Michigan. J. Parasit. **29:** 182–186.

HERBER, E. C. 1942 Life history studies on two trematodes of the subfamily Notocotylinae. J. Parasit. **28:** 179–196.

HOPKINS, S. H. 1938 A new heterophyid cercaria from Texas. J. Parasit. **24** (Suppl.): 26.

JONES, T. W. T. 1951 A new monostome cercaria from bithynoid snails in Singapore. Parasit. **41:** 312–315.

KUNTZ, R. E. 1952 Embryonic development of the excretory system in a pleurolophocercous (acanthostomid) cercaria, three stylet cercariae (a microcercous cercaria, a brevicaudate, and a longicaudate dicrocoeliid cercaria) and in a microcaudate eucotylid cercaria. Trans. Amer. Micr. Soc. **71:** 45–81.

LUNDAHL, W. S. 1941 Life history of *Caecincola parvulus* Marshall and Gilbert (Cryptogonimidae, Trematoda) and the development of its excretory system. Trans. Amer. Micr. Soc. **60:** 461–484.

———1955 Notes on collecting trematode-infected Amnicolidae in deep waters. J. Parasit. **41:** 441–442.

MARTIN, W. E. AND KUNTZ, R. E. 1955 Some Egyptian heterophyid trematodes. J. Parasit. **41:** 374–382.

MATHIAS, P. 1925 Recherches expérimentales sur le cycle evolutif de quelques trématodes. Bull. Biol. France et Belgique **59:** 1–123.

ROTHSCHILD, M. 1936 A note on the variation of certain cercariae (Trematoda). Nov. Zool. **40:** 170–175.

———1938 The excretory system of *Cercaria coronanda* n. sp. together with notes on its life history and the classification of cercariae of the superfamily Opisthorchioidea Vogel, 1934 (Trematoda). Nov. Zool. **41:** 148–163.

SEWELL, R. B. S. 1922 Cercariae Indicae. Ind. J. Med. Res. **10:** Supp.

ULLMAN, H. 1954 Observations on a new cercaria developing in *Melanopsis praemorsa* in Israel. Parasit. **44:** 1–15.

WESENBERG-LUND, C. 1934 Contributions to the development of the trematoda digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. Danske Vidensk. Selsk. Skr. Naturv. og Math. Afd. **5:** 1–223.

EXPLANATION OF PLATE

FIG. 1. *Cercaria amnicolensis,* ventral aspect; camera lucida.

FIG. 2. *C. amnicolensis,* optical section of oral sucker showing the buccal cavity, lateral aspect; camera lucida.

FIG. 3. Redia of *C. amnicolensis* containing immature cercariae; projection.

FIG. 4. *Cercaria parapleurolophocercoides,* ventral aspect; camera lucida.

FIG. 5. *C. parapleurolophocercoides* redia containing immature cercariae; projection.

FIG. 6. *C. parapleurolophocercoides,* en face view of body showing lateral caudal fin-folds; camera lucida.

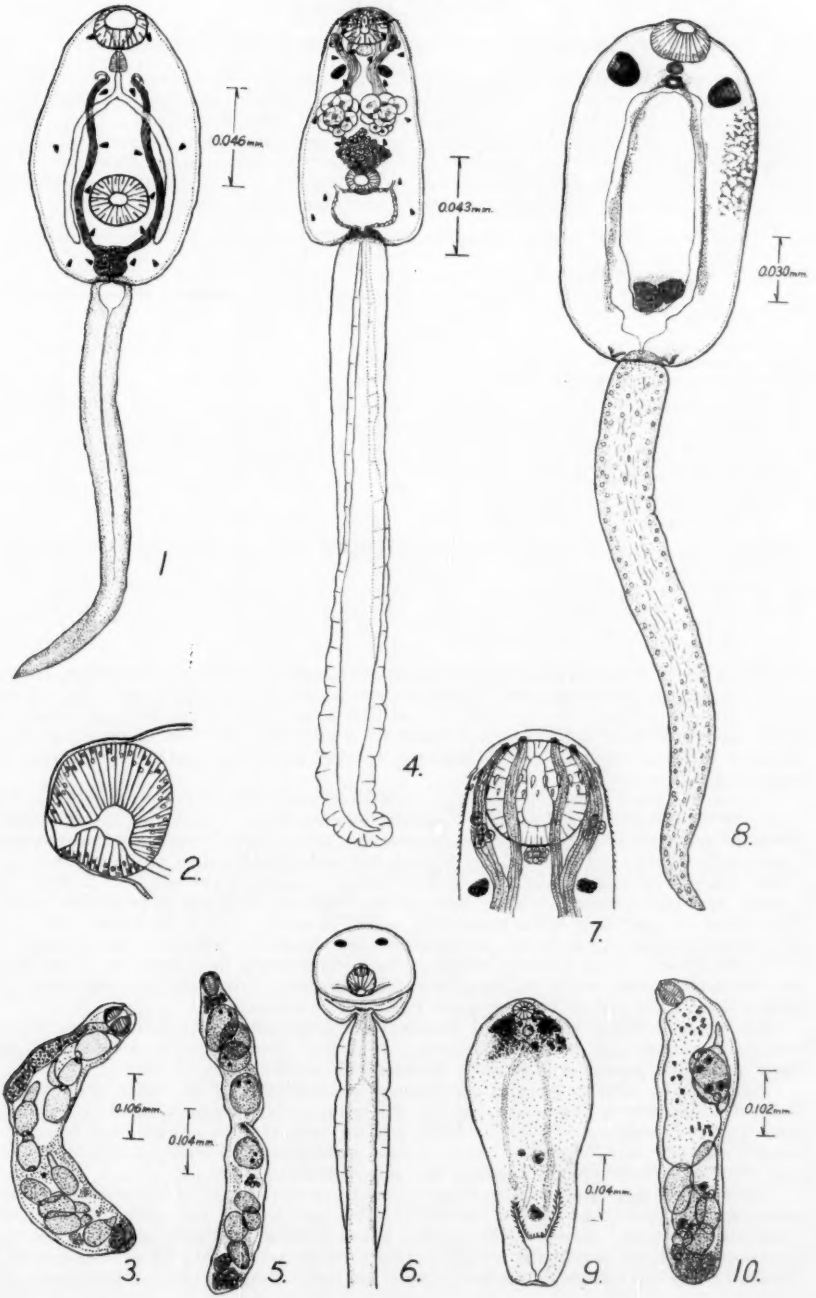
FIG. 7. *C. parapleurolophocercoides,* dorsal aspect of anterior end showing cephalic glands, their ducts and orifices (6-4-4-6), and cuticular spines; free-hand.

FIG. 8. *Cercaria wesenberg-lundi,* ventral aspect; camera lucida.

FIG. 9. Immature adult of *C. wesenberg-lundi,* from experimental infection in chick 4 days after ingestion of cysts, ventral aspect; camera lucida.

FIG. 10. Redia of *C. wesenberg-lundi* with immature cercariae; projection.

PLATE I


0.046mm.
1
0.043mm.
4.
0.030mm.
8.
2.
7.
0.106mm.
0.104mm.
3.
5.
6.
0.104mm.
9.
0.102mm.
10.

RESEARCH NOTE

INTESTINAL PARASITES IN KATHMANDU, NEPAL

During a stay in Kathmandu, May and June, 1955, it was possible to conduct a brief survey in the city and its environs. Of the adult population only 7 hospital patients and 11 staff members and servants in the St. Xavier boarding school for boys could be examined. All the others were children, mostly under 10 years of age. All but 8 were boys. A single stool specimen in saline and in iodine solution was examined from each person. No permanent staining could be made and no concentration methods were used. While facilities were such that it was necessarily an incomplete survey, it furnished, nevertheless, some illuminating results (Table I).

TABLE I.—*Intestinal parasites found in single stool examinations, Kathmandu, Nepal*

	Adults	Peasant children	Upper class children
	a	b	c
No. persons examined	18	44	102
Parasites found	%	%	%
Entamoeba histolytica (c)*	22	9	5
E. coli (c)	22	18	8
Endolimax nana (c)	28	5	3
Iodamoeba butschlii (c)	11	7	6
Giardia lamblia (c)	0	18	3
Chilomastix mesnili (c)	6	0	1
Trichomonas hominis (m)**	6	0	0
Enteromonas hominis (m)	0	2	0
Compound incidence of *Entamoeba histolytica, E. coli* & *Endolimax nana*	24	24	5
Difference	a–c = 19 ± 6%	a – b = 13 ± 6.4%	b – c = 6 + 3%
Ascaris lumbricoides	6	70 ± 7%	26 ± 4.4%
Trichuris trichiura	28	20	15
Hookworm	0	9	3
Abnormal consistency of stool	28	45	7

* c) cysts ** m) motile forms

Protozoa.—In the adult group 8 were examined because of abdominal complaints, the remaining 10 because they belonged to the staff and servants of the boarding school. The children were examined regardless of any complaint, solely as being pupils in one boarding school for chiefly upper class boys and in one day school for peasant children. The adult group, being small and somewhat selected, must be treated with some reservation, especially regarding the incidence of *E. histolytica.*

In an earlier study in Sweden (Svensson, Acta Med. Scand. Suppl. [10], 115 pp., 1935 it was shown that the compound incidences for *E. coli, E. histolytica* and *E. nana* give figures comparable in different surveys conducted on the one-specimen basis. Table I shows that the boarding school pupils (upper class children) are less infected with protozoa than peasant children and adults. The difference in compound incidences is in accordance with expectations. In Nepal, as in many other places, peasants are not as a rule satisfactorily cleanly in their personal habits. The children are very early left to themselves, to play about just outside the houses, where the younger children also regularly pass their stools. The children of well-to-do parents are well looked after, mostly having a woman servant entirely occupied with their care. As to the infection with contaminated food it can be estimated as moderate. Few things are eaten raw, and the food is not much touched by other hands than those of the eater.

The higher incidence in the adults than in the children also conforms to the experience from earlier surveys and general observations. Protozoal infections are, as a rule, very persistent and their frequency will naturally increase with increasing age.

Regarding the parasites found, those common in other parts of the world occur also in Nepal. Of *Dientamoeba fragilis* no estimation was made, as this amoeba cannot be identified in ordinary wet preparations. *E. coli,* as usual, was the most common species, with *Iodamoeba bütschlii* relatively so. Flagellates, except *Giardia* in the peasant children, seemed to be comparatively rare. Purging might, however, have provided more positives.

Helminths.—The peasant children showed a much higher incidence of *Ascaris,* and perhaps hookworm, than upper class children and adults. This accords with their greater opportunities of acquiring infections. Moreover, the boarding school children are greatly protected from new infections, and are also occasionally treated, if *Ascaris* has been detected by the passing of worms. The adult group had the low incidence of *Ascaris* commonly observed with increasing age. Of

special interest is the high incidence of *Trichuris* in all 3 groups, emphasizing again that infections with this species are very persistent when once acquired. It should be noted that *Schistosoma* was not found and according to local doctors it does not occur in the Kathmandu region. Living conditions and agricultural methods are such that *Schistosoma* infections might have been expected if the snail intermediate hosts occur.

The consistency of the stools was observed and recorded. Loose, liquid and mucus containing stools were designated as abnormal. Hard stools were rare. The figures for the adults group may be left without consideration, as a high percentage was examined because of intestinal trouble. The boarding school group had a low incidence of abnormal consistency. Microscopically their stools showed that the food had been well digested. The peasant children had not less than 45% of stools of abnormal consistency. This did not seem to inconvenience them to any appreciable degree and on the whole they seemed quite healthy. Probably the looseness of stools only means that their ingesta pass through the colon in an unusually short time. That higher parts of the intestine are not involved is indicated by the fact that the food was generally well digested and that motile forms of protozoa were rarely encountered.—RUTH SVENSSON, *Kathmandu, Nepal* (Address: *Norrlandsgatan 12, Uppsala, Sweden*).

ERRATA

Page 549, vol. 41 (5) under "Procedure" parts of the second and third sentences have been omitted. These sentences should read: "Extraneous material was removed by washing with tap water, and cestodes which had settled were removed with a rubber bulb pipette. Two to four gravid worms were selected and placed on a microscope slide with a drop of water."

Vol. 41 (6, sec. 2): 43 (Abs. 107): a) 5th line, 2nd paragraph—"grain" should be "gain." b) next to last line—insert "feed" between "better" & "utilization."

PRELIMINARY ANNOUNCEMENT OF THE 1956 MEETING

The 31st Annual Meeting of the American Society of Parasitologists will be held on the campus of the University of Connecticut, Storrs, Connecticut, from August 26 to 30, 1956. Inasmuch as the deadline for program arrangements is June 1, 1956, all titles and abstracts of papers to be presented at Storrs must be in the hands of the Secretary not later than May 25, 1956. By Council action, each member is entitled to not more than 15 minutes to present his papers. A paper by a group of authors is credited against the time allotted to the individual presenting the paper so that no one individual may use more than the 15 minutes allotted. A crowded program may necessitate cutting the allotted time to a minimum of 10 minutes. Please plan to present your papers by demonstration if at all possible. Blanks for submitting titles and information regarding housing will be sent out to all members as soon as the information becomes available.

A. C. Walton, Secretary
Knox College, Galesburg, Illinois

Dates for Mailing of Numbers of Volume 41, (1955).

No. 1. February 1955—Mailed March 1, 1955
No. 2. April 1955—Mailed April 13, 1955
No. 3. June 1955—Mailed June 16, 1955
No. 4. August 1955—Mailed August 12, 1955
No. 5. October 1955—Mailed October 12, 1955
No. 6. December 1955—Mailed December 15, 1955

25-YEAR INDEX

(Covering volumes 1–25, 1914–1939)

of the

Journal of Parasitology

Over 9000 Entries, Subdivided

AUTHOR—SUBJECT—HOST

Included in the 3397 author entries are 1141 from abstracts of papers on the first 15 annual programs of the American Society of Parasitologists (1925–1939), and 944 from notes of Proceedings of the Helminthological Society of Washington, meetings 16–156 (Dec. 18, 1913–May 20, 1933). A special subdivision of the subject index is a 10-page list of the 684 new species of animal parasites described (including 76 new genera), made bibliographic for the whole text of the 25 volumes. The host index contains 1645 entries, 364 of them referring to man.

Students and investigators will find this perspective of a quarter century of American parasitological publication an invaluable reference work, not only for their own desks but for their institutional libraries. Copies may be obtained at $2.00 each from the Custodian, Wilford S. Bailey, American Society of Parasitologists, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama.

BIOLOGICAL ABSTRACTS
Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in five low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section C—Abstracts of Microbiology, Immunology, Public Health, and Parasitology—affords a very complete coverage of the Parasitologists' literature. The list price is $18.00 a year, less 20% discount to non-profit, educational institutions and 50% to individuals. Foreign subscribers add 50 cents for postage.

Write for a sample copy.

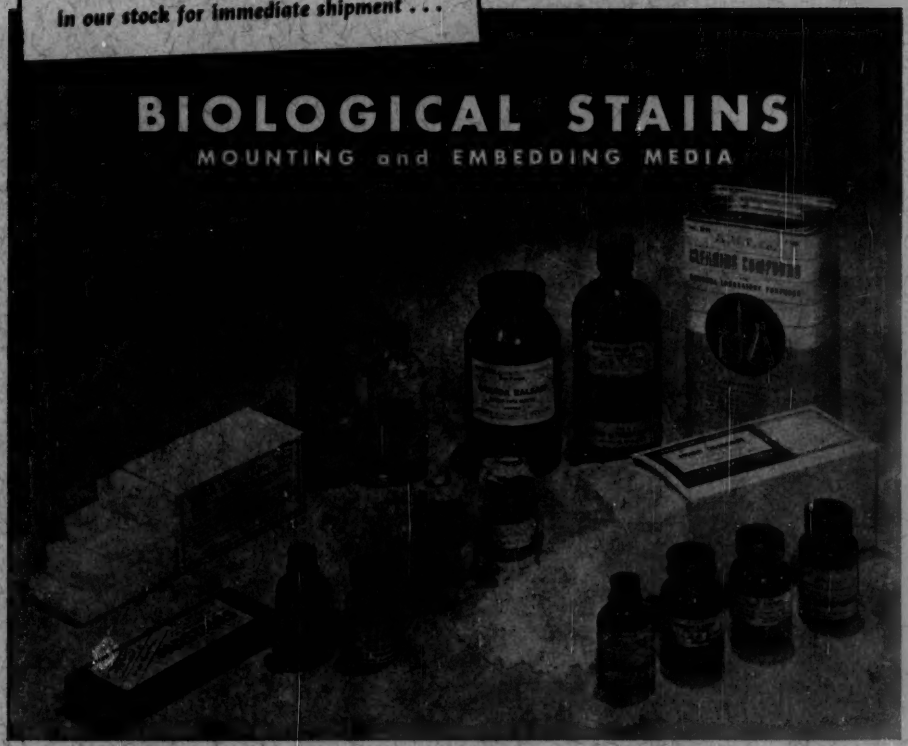
BIOLOGICAL ABSTRACTS

University of Pennsylvania
Philadelphia 4, Pa.

An assortment of 700 items
in our stock for immediate shipment . . .

BIOLOGICAL STAINS

MOUNTING and EMBEDDING MEDIA



The brands listed below have been selected for purity, dependability and responsibility of the maker. Our prices are identical with those charged by the manufacturer.

BIOLOGICAL STAINS, DRY
- Coleman & Bell Dry Stains
- Harleco Dry Stains and "Parstains"
- National Dry Stains

BIOLOGICAL STAINS IN SOLUTION
- Coleman & Bell Stains in Solution
- Harleco Stains in Solution
- Hynson, Westcott & Dunning Giemsa Solution

MOUNTING MEDIA FOR MICROSCOPY
- A.H.T. Co. Canada Balsam
- Coleman & Bell Balsam, etc.
- Flatters & Garnett "Euparal"
- Harleco Resins, etc.

EMBEDDING MEDIA FOR HISTOLOGY
- A.H.T. Co. Paraffin
- Fisher "Tissuemat"

Copy of Reagent catalogue sent upon request.

SYMBOL OF QUALITY
A.H.T.CO.
SPECIFICATION
LABORATORY APPARATUS

ARTHUR H. THOMAS COMPANY

Laboratory Apparatus and Reagents

WEST WASHINGTON SQUARE **PHILADELPHIA 5, PA.**

Teletype Services: Western Union WUX and Bell System PH-72

CONTENTS

JOURNAL OF PARASITOLOGY

Volume 42, Number 1 February 1956

PARASITISM AND PARASITOLOGY. Clay G. Huff (1–10)

ELECTRON MICROSCOPE OBSERVATIONS OF *Typanosoma equiperdum*. Everett Anderson, L. H. Saxe and H. W. Beams (11–16)

THE EFFECT OF HOST CASTRATION AND FASTING ON THE RATE OF GLYCOGENESIS IN *Hymenolepis diminuta*. Jack W. Daugherty (17–20)

PROTEOLYTIC ACTIVITY IN EXTRACTS OF THE ESOPHAGUS OF ADULTS OF *Ancylostoma caninum* AND THE EFFECT OF IMMUNE SERUM ON THIS ACTIVITY. Ralph E. Thorson (21–25)

THE EFFECT OF EXTRACTS OF THE AMPHIDIAL GLANDS, EXCRETORY GLANDS, AND ESOPHAGUS OF ADULTS OF *Ancylostoma caninum* ON THE COAGULATION OF DOG'S BLOOD. R. E. Thorson (26–30)

OBSERVATIONS ON THE PLEROCERCOID LARVA OF *Pelichnibothrium speciosum* MONTICELLI 1889. Nathan W. Riser (31–33)

OCCURRENCE OF THE CESTODE *Mathevotaenia surinamensis* (COHN 1902) SPASSKII 1951 IN A NORTH AMERICAN ARMADILLO. G. D. Buchanan (34–38)

A NEW SPECIES OF *Centrorhynchus* (ACANTHOCEPHALA) FROM THE KITE, *Milvus migrans*, IN EGYPT. Helen L. Ward (39–41)

Lophortofilaria californiensis N.G., N. SP. (FILARIOIDEA, DIPETALONEMATIDAE) FROM CALIFORNIA QUAIL, *Lophortyx californicus*, WITH NOTES ON ITS MICROFILARIA. Everett E. Wehr and Carlton M. Herman (42–44)

TROGLOSTRONGLYLUS WILSONI (STOUGH, 1953) N. COMB. (NEMATODA: METASTRONGLYLIDAE) FROM THE LUNGS OF THE BOBCAT, *Lynx rufus rufus*. Luz Sarmiento and B. D. Stough (45–48)

NIDIFICATION OF A MITE (*Psorergates simplex* TYRRELL, 1883: MYOBIIDAE) IN THE SKIN OF MICE. Robert J. Flynn and Bernard N. Jaroslow (49–52)

A NEW SPECIES OF *Coelomomyces* (FUNGI) FROM TASMANIAN MOSQUITO LARVAE. Marshall Laird (53–55)

Probolitrema mexicana, N. SP., AN ANAPORRHUTINE TREMATODE FROM ELASMOBRANCHS OF BAJA CALIFORNIA. Edward K. Markell (56–59)

Myocheres inflata A NEW SPECIES OF PARASITIC COPEPOD FROM THE BAHAMAS. J. A. Allen (60–67)

A REDESCRIPTION OF *Crusia americana*, A NEMATODE PARASITIC IN THE OPOSSUM, *Didelphis marsupialis Virginiana*. John L. Crites (68–72)

ECTOPARASITES FROM SWALLOW NESTS, WITH THE DESCRIPTION OF A NEW CERATOPHYLLID FLEA. Richard B. Eads (73–76)

IDENTIFICATION OF BETA ZnS IN THE INTESTINAL CELLS OF *Strongylus* SPP. David T. Clark (77–80)

THE LOCATION OF THE SCHISTOSOME VECTORS, *Australorbis glabratus* AND *Tropicorbis centimetralis*, ON AND IN THE SOIL ON DRY NATURAL HABITATS. Louis Olivier (81–85)

THREE NEW CERCARIAE FROM *Amnicola pilsbryi* WALKER, 1906, WITH SOME NOTES ON THEIR LIFE HISTORIES. Frank J. Etges (86–93)

RESEARCH NOTES:

Ophiotaenia grandis LA RUE (CESTODA: PROTEOCEPHALIDAE) IN MCCURTAIN COUNTY, OKLAHOMA: **Larry S. Roberts, 20.** SOME HELMINTHS OF OREGON URODELES: **Donald L. Lehmann, 25.** NOTES ON THE USE OF TECHNICON MOUNTING MEDIUM FOR IN TOTO PREPARATIONS: **Frank J. Etges, 30.** *Hoplopleura oenomydis* FERRIS IN PUERTO RICO: **Irving Fox, 33.** THE PIG AS A HOST OF *Schistosoma mansoni* IN PUERTO RICO: **G. T. Riggin, Jr., and Luis A. Berrios, 10.** INTESTINAL PARASITES IN KATHMANDU, NEPAL: **Ruth Svensson, 94.**

ERRATA (95)

ANNOUNCEMENT (95)

DATES FOR MAILING ISSUES OF VOLUME 41 (1955) (95)

BUSINESS PRESS, INC.
LANCASTER, PA.